POST-IRRADIATION DEVELOPMENT OF CHROMOSOMAL DAMAGE IN SEEDS

By

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INTRODUCTION

A considerable body of data has accrued and a number of significant hypotheses have been put forward in the last ten years concerning the radiobiology of seeds. Much of this work has been based solely or predominantly upon a single biological criterion, viz, seedling height. Justification for extension of results obtained in such studies to the consideration of other biological responses and to the mechanisms possibly operative in effecting them rests on correlative studies of the various criteria of radiation effect.

The present study was undertaken to investigate to what degree seedling height reduction (length of first leaf) in barley is related to the presence of chromosomal aberrations in the cells of the embryonic root meristems of irradiated seeds. The effect of water content, storage, and oxygen tension upon this correlation is examined on an individual plant basis.

REVIEW OF LITERATURE

Plant seeds and such dry fruits as the grains of cereals (which are commonly referred to as seeds) have been recognized as constituting remarkably useful material for a broad spectrum of biological studies. This review will be restricted to radiation effects and their modification especially as studied in barley.

Early Work

The pioneer work of Stadler (1928a,b) establishing the mutagenic effect of X rays and gamma rays on plants, and paralleling that of Muller (1927) with Drosophila, was largely done with barley. Reading these papers after thirty-five years one cannot help but be impressed by the number of significant observations and hypotheses arrived at so early. By 1930, Stadler (1930) was able to summarize his research and draw a number of important, if tentative, conclusions, explicit or implied, only some of which are cited below:

1. Induced mutations are similar to spontaneous ones.
2. Induced mutations are almost exclusively recessive.
3. Induced mutations are almost exclusively deleterious.
4. Induced mutations may in many cases be deletions.
5. Mutation frequency increases linearly with dose.
6. Mutation frequency is independent of X ray wave length.
7. A threshold effect may exist for mutation induction.
8. Radiosensitivity (mutation rate) is not affected by moisture content.

9. Radiosensitivity is not affected by temperature.
10. Radiosensitivity (inviability) increases with moisture content.
11. Radiosensitivity (inviability) increases with post-irradiation storage.
12. Radiation causes chromosomal disturbances.
13. Radiation imposed during early development is a useful tool for studying ontogenesis and morphogenesis as well as gene action.

A very impressive list of accomplishments indeed!

Sweden early became and has remained one of the major centers of seed radiation research. Chief credit for this can be attributed to the early and continued research of Ake Gustafsson. In a series of papers (Gustafsson, 1937a, b, 1938) he summarized the results of his early radiation experiments with barley. Most relevant to the work considered here are his observations on frequency of "disturbed cells" (i. e. those cells in division which show chromosomal aberrations) following X irradiation and ancillary treatments.

He found a definite increase in frequency of disturbed cells with increase in post-irradiation storage. This he termed "cytological after-effect" (Gustafsson, 1937a, p. 326). None of his material was germinated immediately after irradiation, but the proportion of dividing cells showing aberrations when stored 18 days was almost double that stored 4 days. In the same paper he reported that higher water content is associated with a higher frequency of disturbances. It should be noted, however, that his driest seeds were approximately 10% water and that the comparison was made with seeds which were soaked for varying periods of time to increase their water content.

Gustafsson (1937b) maintained that following the X irradiation of the resting barley seed, the prevalance of disturbed cells was greater in certain regions of the roots upon germination. He attributed this to increased cellular activity which in turn might be brought about by higher water content in those cells at the time of irradiation. He also found greater frequency of disturbances in irradiated aged seeds.

Gelin (1941), examining material from the same treatments employed by Gustafsson, modified and extended Gustafsson's conclusions. He found no real difference in the various histogens of the roots as regards frequency of disturbed cells and therefore, combined the data from these different layers. The summary table in which he compared his cytological findings with the sterility and mutation data from Gustafsson (1940) is reproduced here in simplified form.

Golden Barley 1939 Harvest

Irradiated 160 KV, 7.5 ma 4 mm Al 72 r/min Dose 10 kr

Water Content	Disturbed Cells	Sterility X_1	Mutation X_2
10%	12.66%	51.6%	7.9%
15%	27.97%	76.7%	13.4%
Soak 23 hours	53.80%	87.6%	26.2%

Gustafsson (1940, p. 7) had concluded that "Sterility and mutation imply connected phenomena." The above results formed the basis for Gelin's assertion that an even closer correlation exists between disturbed cells and mutation rate in the X_2.

Froier and Gustafsson (1944) demonstrated that the length of first leaf and survival in the field increased directly with size of embryo in irradiated wheat. They screened grain from each of two varieties into four size classes and irradiated, at approximately 12% seed

water content, with 10 or 29 kr. They also showed a rather surprising radiation protection effect provided by the presence of hulls in barley and oats. The objection might be leveled that the barley used in their study was of different varieties but in the case of oats, the hulless condition was obtained by mechanical removal and there was no genetic difference.

Luther Smith (1951), in his extensive review of the cytology and genetics of barley, summarized en passant most of the early radiation work. It will only be noted here that although search for fundamental principles was not neglected, as evidenced by much of the foregoing, the chief impetus behind many of the earlier investigations was the desire to obtain a maximum yield of induced mutations for breeding work. Stadler (1930) had clearly indicated that mutation induction by radiation was not likely to prove a shortcut to success in this applied field except in rare circumstances, but when no dramatic success was immediately forthcoming, the whole field of radiation botany progressed only slowly until after World War II.

The Nuclear Age

The atomic bombs which ended the war and their continued testing, with the consequent increase in radioactive fallout, created a great resurgent interest in radiobiology which has been paralleled by a rapidly expanding technology. These have led to accelerated activity not only in the applied field but in fundamental research as well: witness the two International Conferences on the Peaceful Uses of Atomic Energy (United Nations 1955, 1958).

Moisture effect

Among recent developments in the radiobiology of seeds has been

the discovery that seeds drier than the "normal" state, which is about 10 to 12% water, evince an inverse relationship between moisture content and radiosensitivity based either on seedling height (Ehrenberg and Nybom, 1954; Caldecott, 1954, 1955a) or on chromosomal aberrations in the germinating shoot tip (Caldecott, 1955b). Concerning this latter relationship, Caldecott showed that for seeds of different water contents all receiving 20 kr of X irradiation there was a sharp rise in per cent normal cells (those anaphases not showing bridges or fragments) from embryos with 4% to those with 8% water, at which point the effect of moisture on cytological damage leveled off. The actual values obtained were 4.0% and 27.9% normal cells respectively.

More recently, Conger (1961) has reported that for "super dry" seeds (less than 3% water) there is a decrease in radiosensitivity (based on seedling height) which parallels the decrease in the number of long-lived free radicals as determined by electron paramagnetic resonance.

After-effect

The last paper cited above deals not only with water content but with the influence this has during post-irradiation storage, i. e. the "after-effect" phenomenon of Gustafsson as mentioned previously and which recurs sporadically throughout the early literature (Tascher, 1929; Wertz, 1940; Sax, 1941).

Kaplan (1951) and Caldecott and Smith (1952) observed that heat treatments applied after irradiation was completed could alter the response. The latter authors reported increased mutation rates and seedling heights, reduced chromosomal aberrations both in root tips and in microsporocytes, and no significant alteration of survival to

maturity with treatment of 75, 80, or 85° C for 30 minutes after irradiation.

Adams et al. (1955) demonstrated a long term after-effect phenomenon in irradiated barley in contrast to the short term responses cited above which had also been reported for other organisms and for organic compounds as well (cf. Mitchell and Holmes, 1954). Using seeds with approximately 8% water content, an X ray dose of 7.5 kr and storage times of 2, 4, 6 and 8 weeks, they found an approximate 80% increase in bridges per cell, and a 60% decrease in seedling height and in germination at the longest storage time when in oxygen. The after-effects were less when storage was in air or nitrogen. They were progressive with storage time but showed a tendency toward leveling-off after 6 weeks.

This work confirmed and extended the observation of Ehrenberg (1955a) that post-irradiation storage of barley causes reduced growth. Lawrence (1955) noted reduced germination and survival in stored x-irradiated barley.

Curtis et al. (1958) in carefully controlled experiments carried out in air, established that the after-effect increases as water content decreases from 12% to 4% (the lowest value tested). They distinguished two components, a brief one which is effective for only 4 hours post-irradiation and is very moisture sensitive, and an extended one which is much less sensitive to moisture content and continues to develop for a month or longer. For seeds with 4% water content, storage for one week post-irradiation increased damage by as much as a factor of twenty over those which received the same dose that were immediately soaked and germinated. This study was based solely on seedling height reduction as a measure of radiosensitivity.

Oxygen effect

Work on the effect of oxygen prior to the realization of the significance of after-effects and of moisture content lacks the control of variables necessary for a clear interpretation and must be considered in this light.

Hayden and Smith (1949) had observed that when germinating barley was irradiated in a partial vacuum, radiosensitivity as measured by chromosomal aberration frequency and by seedling height reduction was not as great as when irradiation was in air. Nilan (1954) found that seedling height, survival, and mutation rate were unaffected by differences in oxygen tension during irradiation but that cytological effects were sensitive. Adams and Nilan (1958) found that although post-irradiation storage under 100 pounds of oxygen increased radiosensitivity as determined by germination, chromosomal aberrations in shoot tips, seedling height and survival in the field, it did not alter mutation rate.

A recent review of the post-irradiation oxygen effect and summary of their own work has been given by Nilan, Konzak, et al. (1961). Of particular note are the following conclusions which they drew. Freezing seed at dry-ice temperature (-78° C) suspends all after-effects until thawing. Other things being equal, the magnitude of the oxygen effect post-irradiation depends on the criterion of radiosensitivity which is used. For reduction in seedling height this is eightfold, for fragments per cell in M_1 shoot tips this is sevenfold, and for chlorophyll mutations per 100 M_2 seedlings this is sixfold. For seeds below 3% water content they find reduction in the after-effect. It becomes negligible in some treatments at about 1% water content.

Caldecott (1961; cf. Bozzini, Caldecott and North, 1962) has summarized results he has obtained with seeds of low moisture content (about 4% water in the embryo). Of interest is his finding that post-irradiation storage for 8 days in air resulted in skewed seedling height distributions--even bimodality. Separating the seedlings into three height classes and examining them for interchange frequency in X_1 spikes and for mutant seedlings in the X_2, he found a close correspondence of these criteria with each other and with seedling height reduction. Wolff and Sicard (1961) reported data on "super dry" (about 2% water) and "normal dry" (about 10% water) seeds at variance with the results of Caldecott and of Curtis reported above. Their "super dry" seed was obtained by two months' storage over calcium chloride and their normal dry seed was open-stored in the laboratory. When the super dry seed was stored post-irradiation over the desiccant for varying periods of time up to 32 days, there was no increase in the radiosensitivity as determined by reduction in the mature length of the first leaf. If, however, similar "super-dry" seed was stored under room conditions, it grew even taller than "normal-dry" seed irradiated and stored at room conditions. On the other hand, "normal dry" seed if stored in the desiccator after irradiation showed progressively more after-effect with time--giving a growth response essentially equal to that of the "super-dry" desiccator-stored seed from 18 days onward.

Conger and Fairchild (1952) demonstrated that oxygen tensions greater than that of a normal atmosphere can cause chromosomal aberrations in dry pollen and microspores of Tradescantia which are identical to those caused by ionizing radiation. Ehrenberg et al. (1957) produced similar effects by exposing barley grains to 60 atmospheres

of oxygen for one and two week intervals. Adams and Nilan (1958) did not observe any effect with oxygen at one atmosphere. More recently several research groups have reported mutation induction by oxygen treatment of barley grains (Kronstad et al., 1959; Moutschen-Dahmen et al., 1959).

There is a great miscellany of observations published which touch on the present work in one respect or another and which have not been reviewed here. Some will be dealt with in the discussion section. Two general works on seeds are those authored by Barton (1961) and edited by Stefferud (1961). Numerous reviews and symposia have a bearing on the present work. The most relevant are listed below:

Radiation Protection and Recovery, Hollaender, A. ed. (1960)

Symposium on the Effects of Ionizing Radiations on Seeds (1961)

Symposium on Mutation and Plant Breeding (1961)

Fundamental Aspects of Radiosensitivity, U. S. Brookhaven National Laboratory (1961)

Radiation-induced Chromosome Aberrations, Wolff, S. ed. (1963)

Publications before 1955 are listed in Bibliography on The Effects of Ionizing Radiations on Plants, Sparrow, A. H., J. P. Binnington and V. Pond (1958).

MATERIALS AND METHODS

The seeds

The barley grains used in these experiments were of Hordeum vulgare L. cultivar Himalaya (C. I. 620) from the selected strain maintained by C. F. Konzak, Washington State University. All seeds were from the 1961 harvest.

Upon receipt the seeds were passed through a series of sieves. The major fraction passed through U. S. Standard Sieve Series No. 6 (openings measuring 3360 microns or 0.132 inch) but not No. 7 (2830 microns or 0.111 inch). Only this portion was used. All apparently defective seeds (broken, misshapen or discolored) were removed before storage and the seeds were again checked before use.

The seeds were stored in desiccators over dry calcium chloride from the time they were received until several months prior to use, at which time they were transferred to desiccators maintained at the appropriate relative humidity by dry chemicals or saturated solutions. The various humidities, the means whereby they were obtained, and the moisture content of the seeds are summarized in Table 1. When irradiated, all seeds, regardless of treatments, were in a state of low physiological activity ("resting") but not dormant (i. e. requiring "after-ripening" before germinating).

Special seed treatments

In those experiments designed to study the "oxygen effect," seed

TABLE 1

WATER CONTENT, AT EQUILIBRIUM, OF BARLEY GRAINS STORED AT DIFFERENT CONSTANT HUMIDITIES

Determinations from these experiments, and from the literature.

Storage Over: (at 20°C)	Relative Humidity (at 20°C)	Per Cent Weight Loss (Per Cent Water) Whole Seeds* (130°C for 20 hrs.)	Embryo** (105°C, time unspecified)	Endosperm** (105°C, time unspecified)
P_2O_5 (evacuated)	0***	2.8		
Dry P_2O_5 (not evacuated)	0	3.8	4	5
Sat'd NaOH	6.5	6.7		
Sat'd $ZnCl_2$	10	7.3		
Sat'd $KC_2H_3O_2$	20	9.2		
Sat'd $CaCl_2$	32	10.1	6	8
Sat'd $NaClO_3$	75	15.5	11	14

*Determination following the method of Hart et al. (1959)

**Data from Caldecott (1955b)

***A table of constant humidity at given temperatures over a saturated solution of various chemicals is given in Handbook of Chemistry and Physics, 44th Edition, 1962, pp. 2595-2596.

samples were placed in glass ampoules, attached to a glass manifold by means of Tygon tubing and repeatedly evacuated and flushed with the desired gas. In order to be reasonably sure of removing oxygen from the seeds, initial evacuation was of several hours duration at a pressure of 500 microns of mercury or less, as determined by a McLeod gauge.

The gases used were: prepurified nitrogen with a tested concentration of oxygen of 0.5 $\pm$ 0.2 ppm; oxygen; and air admitted via a column packed with silica gel.

The ampoules were filled with the appropriate gas and the Tygon tubing clamped. For nitrogen and oxygen an overpressure, either 40 or 80 mm Hg over atmospheric, was established as a precaution against possible leaks. Without exception every ampoule retained positive pressure during storage.

The irradiation source

The University of Florida Engineering Gamma Irradiator (UFEGI) was used routinely as a source of radiation. This facility (Duncan et al., 1960) consists of a distributed source of cobalt-60 wafers clad in stainless steel and stacked vertically in each of a ring of twelve aluminum tubes. Irradiation is carried out in a centrally situated tube by lowering the specimen to a point at the center of the source. Thus a very uniform field of high intensity gamma radiation is obtained. External shielding is provided by immersion of the entire tube assembly in a pool of water.

The total source originally had an activity of 835 curies and the extrapolated dose rate over the period of these experiments varied from 4.5 to 3.8 kr per minute at the central location which was employed.

All irradiations were carried out at ambient temperature which because of the nature of the source fluctuated only approximately 10° C from 20° C.

Germination and growth

To initiate germination after irradiation the seeds were immersed in water at room temperature for one-half to one hour and were then sown on wet blotting paper in 90 mm petri dishes and covered. Extremely dry seed lots showed impaired viability which was partially overcome by "humidifying" them prior to soaking (except in those treatments sown immediately after irradiation) by placing them over water in a humidity chamber for several hours. Care was taken to ensure that the embryos had free access to air by turning the seeds embryo side up and keeping the water level very low.

Since it was desired that height reduction and chromosomal damage be compared on an individual basis, it was necessary to provide a means of ordering the seedlings in the dishes. This was accomplished by making grids from glass rods of three or four millimeters diameter that would fit inside the dishes and maintain the seedlings in a precise order.

A convenient form for the grid, and one which was used quite successfully, consisted of three parallel rods approximately 15 mm apart fused at right angles to one rod crossing at their midpoint. As many as five seeds can then be placed in each sector of the grid, giving a total of forty seedlings; when arranged "broadside" to each other there is extremely slight danger of displacement during the growth period.

Throughout germination and growth the seedlings were in a room

maintained at 21° C ± 2° C and under continuous illumination by daylight white fluorescent lamps at an intensity of approximately 3200 lux.

It is generally observed that, even for radiation doses which result in 100% lethality, the coleoptile and first seedling leaf usually elongate one to two centimeters when germination is attempted (Moutschen et al., 1956). This "growth" can be attributed to the swelling of the existing cells as a result of the water imbibed, and to a limited amount of metabolic activity (Haber et al., 1961). Conger (unpublished) has determined that under general experimental conditions the mean value of this expansion or "elongation height" is 16 mm for the first leaf in barley and suggests that for purposes of comparing heights of seedling plants relative to controls this base line value logically ought to be subtracted from all measured means. This procedure was followed in all the original data presented here and is referred to as "corrected" mean height.

Root tip collection

Since individual comparisons of growth response and chromosomal damage on the same seed were to be made, it was necessary to collect mitotic material from the seedlings but not to injure the seedling itself. It proved possible to remove several root tips when they were three to five millimeters long without appreciable injury to the seedlings (Tables 2 and 3). The roots attain this length about 24 to 36 hours after sowing, depending on the treatment. This represents the peak of the first division cycle of mitotic activity (Caldecott and Smith, 1952).

At the time of collection some seeds have failed to develop roots

and others have roots too short to collect without danger of excessive damage to the embryo, hence, no roots were collected from such seedlings. Since these seedlings also tend to be shorter than average the mean height of those seedlings examined cytologically is greater than the mean height for the whole population. In an attempt to reduce this bias the "harvesting" of root tips in most of the experiments was timed to coincide with the time at which a maximum number of seedlings had roots of the proper length. Thus, some plants were excluded because their roots were too long and a somewhat larger proportion because their roots were too short. In some experiments root tips were collected from every seedling which developed them. This was accomplished by successive harvests.

The excised roots were put into vials containing a few drops of 0.2% w/v colchicine in aqueous solution and allowed to continue growth for four to five hours and then fixed by adding approximately 5 ml of Carnoy's fixative (6 ethanol: 3 chloroform: 1 acetic acid).

Since it was impractical to attempt to examine all root tips, the vials were stored until after seedling height determination had been made. At that time a sample was selected from the irradiated material. This sample was representative of the full range of heights except for those in the very lowest classes which in Figures 1 and 2 have the following designations and meanings: X, those seeds with no roots or shoots; 0, those seedlings with roots but with first leaf less than 5 mm long; 1, those seedlings with leaves from 5 to 15 mm long; 2, those seedlings with leaves from 15 to 25 mm long. In general, when attempts were made to examine root tips from these very short seedlings, the few division figures that could be found were very aberrant.

In the case of controls, cytological examination was deliberately carried out on a sample which contained a high proportion of those seedlings which were shorter than the mean. This was done in order to enhance the likelyhood of detecting any possible "spontaneous" chromosomal aberrations, since the rate is known to be extremely low.

Cytological method

For cytological examinations the fixed root tips were prepared by the Feulgen method (9 minute hydrolysis in 1N HCl at 60° C followed by staining with leucobasic fuchsin). The root tips were then softened by fifteen to thirty minutes digestion with 5% w/v aqueous pectinase, a slight modification of the procedure suggested by Wolff (1956). The prepared squashes were preserved either as temporary mounts by sealing with dentist's sticky wax (Conger, 1960) or 25 permanent slides by the freeze dry method of Conger and Fairchild (1952) using liquid nitrogen for freezing and diaphane for mounting.

Each root tip was squashed separately and the slides coded. All scorings were made by one observer (the writer) solely on the basis of whether or not any aberration was present in the colchicine-arrested metaphases. In one series of observations scored independently by a second cytologist, the differences in per cent normal metaphases estimated did not exceed 5.

EXPERIMENTAL RESULTS

Appropriateness of the method

In order to investigate the relationship between growth response and chromosomal damage in barley seedling populations arising from grain irradiated and stored under very dry conditions, the usual procedure of subsampling is not appropriate. This is because of the highly heterogeneous, even bimodal, growth response of such seedlings (Figure 1a and 1b) which contrasts sharply with the fairly normally distributed heights of populations obtained in the usual radiation experiments with more moist seeds or those not stored (Figure 2).

Consequently, the method used in these studies consisted of individual sampling of two root tips from seedlings which were then permitted to continue growth until measured for height. (See methods section for details of the procedure.)

First it was necessary to determine whether the removal of several root tips *per se* resulted in any impairment of subsequent growth. Data gathered in the course of experiments designed primarily for other purposes demonstrate rather conclusively (Tables 2 and 3) that under the differing conditions of these experiments there is no effect upon growth during the time period which is involved, namely, seven to nine days.

Secondly, it was important to determine the consistency of aberration frequency between roots from the same seedling.

Barley grains upon germination usually develop a total of seven

FIGURE 1a

FREQUENCY DISTRIBUTION HISTOGRAMS OF SEEDLING HEIGHTS FOR BARLEY SEEDS WITH 2.8% WATER CONTENT; GAMMA-IRRADIATED IN AIR OR IN OXYGEN AT LOW DOSES AND STORED FOR 7 DAYS POST-IRRADIATION

The data illustrate the bimodal distribution of seedling heights which occurs with very dry seed stored after low doses of gamma-irradiation.

Upper figures: seeds irradiated and stored in air over P_2O_5
Lower figures: seeds irradiated and stored in oxygen at one atmosphere positive pressure

Doses were 0, 2, 4, 6, 8 kr Co^{60} gamma-irradiation at 4.25 kr/min, with approximately 75 seeds per treatment. Seedlings were measured after nine days growth.

Height class interval 1 cm centered upon integral centimeters. Zero class indicates seeds which developed roots, but had a shoot less than 5 mm long. The class below this (to the left of zero class) showed no evidence at all of germination and was usually excluded from calculation of means since it showed no relation to dose.

The mean height for each treatment is indicated on each histogram with the conventional symbol $\bar{x}$.

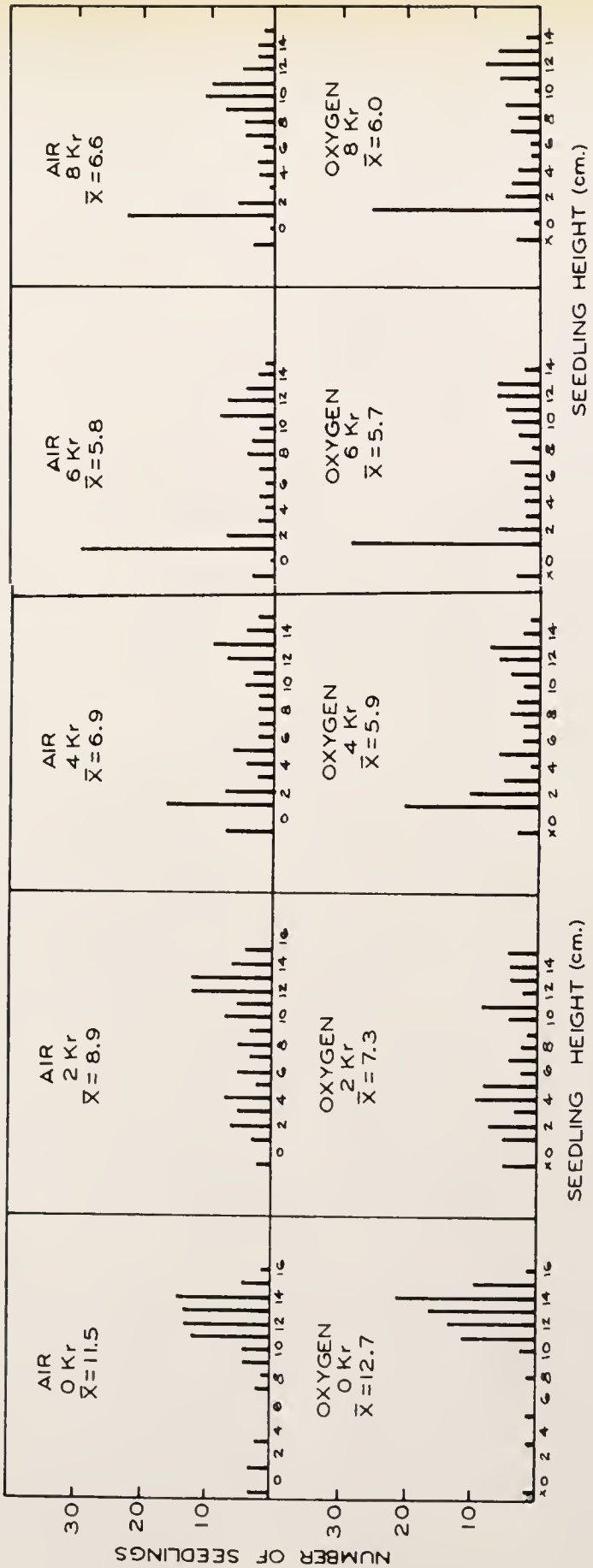

AIR
0 Kr
X̄=11.5
AIR
2 Kr
X̄=8.9
AIR
4 Kr
X̄=6.9
AIR
6 Kr
X̄=5.8
AIR
8 Kr
X̄=6.6
OXYGEN
0 Kr
X̄=12.7
OXYGEN
2 Kr
X̄=7.3
OXYGEN
4 Kr
X̄=5.9
OXYGEN
6 Kr
X̄=5.7
OXYGEN
8 Kr
X̄=6.0
NUMBER OF SEEDLINGS
SEEDLING HEIGHT (cm.)
SEEDLING HEIGHT (cm.)

FIGURE 1b

FREQUENCY DISTRIBUTION HISTOGRAMS OF SEEDLING HEIGHTS FOR BARLEY SEEDS WITH 2.8% WATER CONTENT; GAMMA-IRRADIATED AT HIGH DOSES AND STORED POST-IRRADIATION

The data illustrate that the bimodal distribution of seedling heights which occurs with very dry seed after gamma-irradiation is clearly manifest with a dose of 18 kr but is not present with a dose of 36 kr due to the severity of damage.

Combined data from two experiments which differed only in length of post-irradiation storage in dry air, viz., 3 days and 160 days. The means were nearly the same and the modes were practically identical.

Doses were 0, 9, 18, 36 kr Co^{60} gamma-irradiation at approximately 4.5 kr/min. The total number of seedlings per dose is 241, 238, 228, and 209, respectively. Seedlings were measured after eight days growth.

Height classes are as in Figure 1a. That class designated x showed no signs of germination.

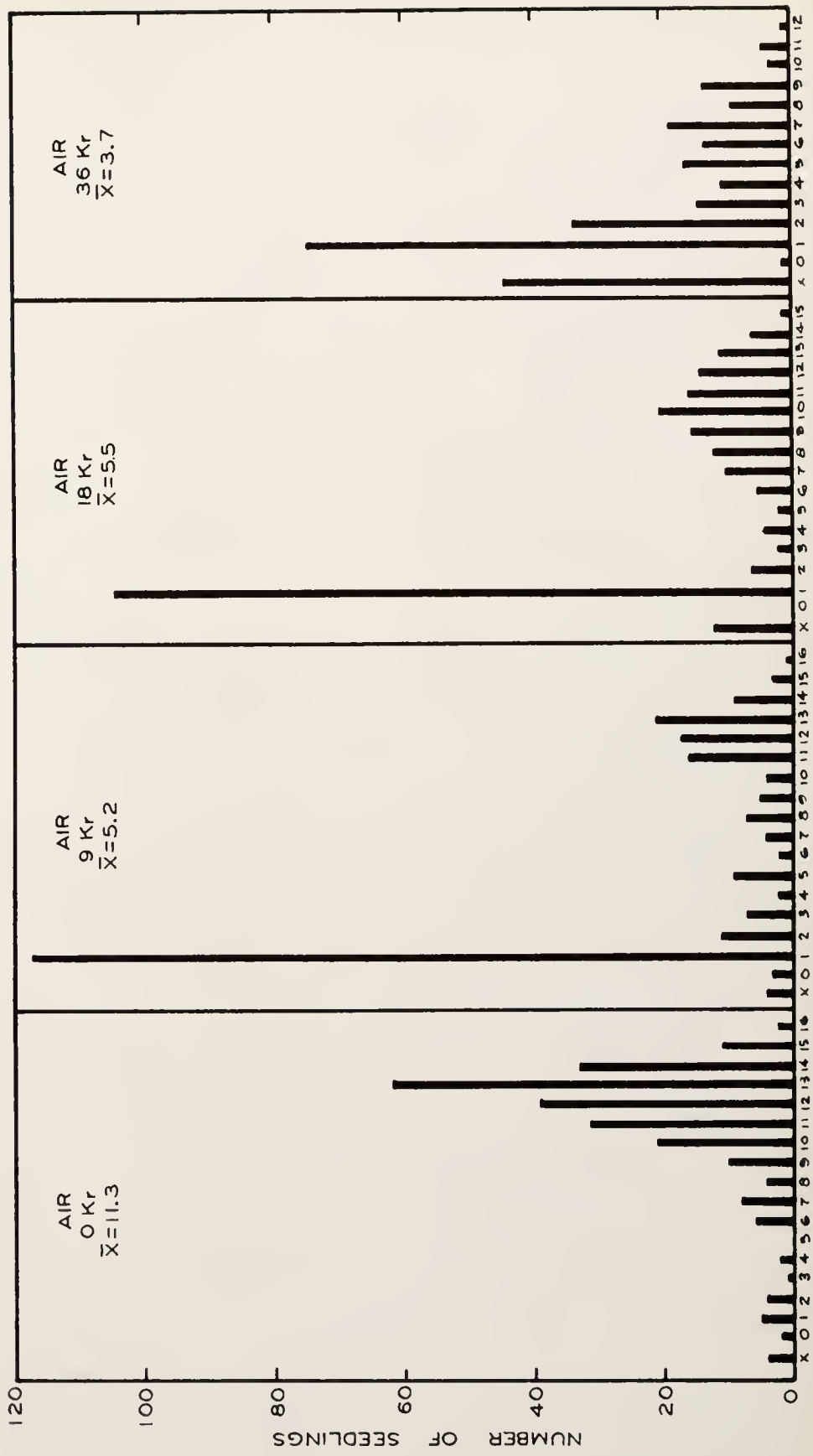

AIR
0 Kr
X̄=11.3
AIR
9 Kr
X̄=5.2
AIR
18 Kr
X̄=5.5
AIR
36 Kr
X̄=3.7
NUMBER OF SEEDLINGS
SEEDLING HEIGHT (cm.)

FIGURE 2

FREQUENCY DISTRIBUTION HISTOGRAMS OF SEEDLING HEIGHTS FOR BARLEY SEEDS WITH 3.8% WATER CONTENT; GAMMA-IRRADIATED IN NITROGEN AND GERMINATED IMMEDIATELY

The data illustrate the essentially normal distribution of seedling heights which is usually found in radiation experiments other than those involving very dry seeds subjected to post-irradiation storage.

Doses were 0, 20, 30 and 50 kr at 3.83 kr/min. The number of seeds per dose were respectively 78, 78, 73 and 77. Seedlings were measured after nine days growth.

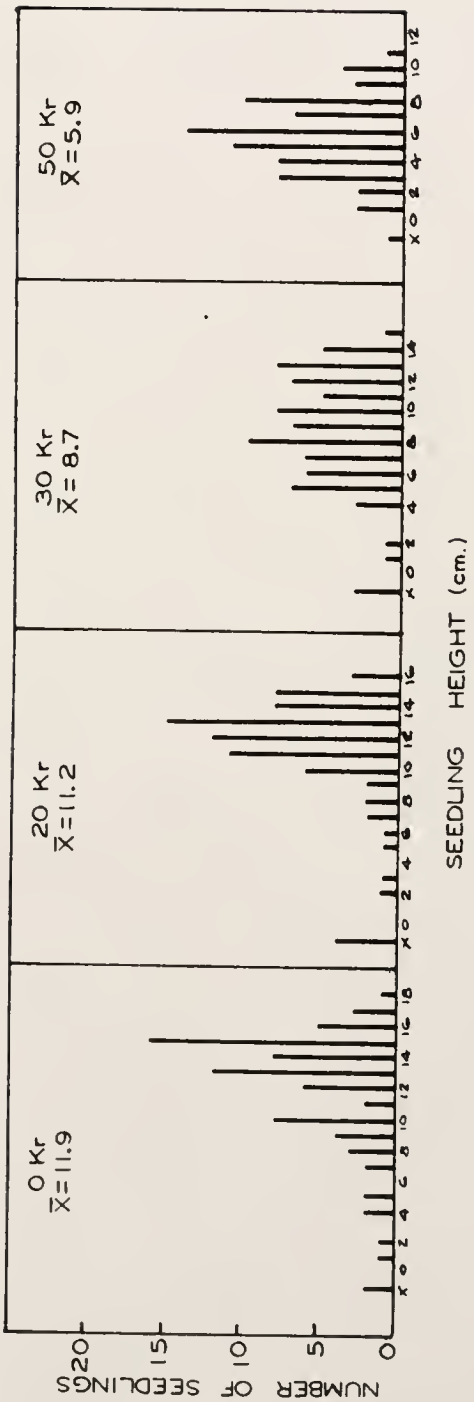

0 Kr
X̄=11.9
20 Kr
X̄=11.2
30 Kr
X̄= 8.7
50 Kr
X̄=5.9
NUMBER OF SEEDLINGS
SEEDLING HEIGHT (cm.)

TABLE 2

COMPARISONS BETWEEN DISHES OF MEAN HEIGHTS AT 8 DAYS OF SEEDLINGS WHICH EITHER HAD TWO ROOT TIPS EXCISED OR WERE LEFT INTACT

Seeds with 2.8% water content gamma-irradiated as specified and stored in air 8 days post-irradiation.

	CUT			UNCUT			
Dose (kr)	Dish	No. of Seed-lings	Mean Height (mm)	Dish	No. of Seed-lings	Mean Height (mm)	Difference (mm) Cut - Uncut
0	1	24	117.9	1	20	114.0	
	2	25	106.4	2	20	108.0	
	3	26	110.0				
	4	26	111.9				
	Treatment Mean		111.5	Control Mean		111.0	0.5
9	1	25	55.6	1	20	48.5	
	2	26	54.2	2	20	60.0	
	Treatment Mean		54.9	Control Mean		54.3	0.6
18	1	24	47.1	1	20	41.5	
	2	25	56.0	2	20	60.5	
	3	26	50.0				
	4	22	50.9				
	Treatment Mean		51.0	Control Mean		51.0	0
36	1	19	27.9	1	19	28.9	
	2	19	31.1	2	19	32.6	
	3	21	35.7				
	Treatment Mean		31.7	Control Mean		30.8	0.9
						Mean	0.5

No statistical test is required to demonstrate the lack of effect of excision of two roots on seedling height.

TABLE 3

COMPARISONS WITHIN DISHES OF MEAN HEIGHTS AT 9 DAYS OF SEEDLINGS WHICH EITHER HAD TWO ROOT TIPS EXCISED OR WERE LEFT INTACT

Seeds with 3.8% water content gamma-irradiated in nitrogen at different doses and either germinated immediately (NI) or stored in nitrogen (NSN), air (NSA), or oxygen (NSO) for 5 days before germination.

Treatment	Dose (kr)	Mean Height (mm) Cut	Mean Height (mm) Uncut	Difference (mm) Cut - Uncut
NI	0	132.5	134.9	- 2.4
	20	115.4	131.1	-15.7
	30	74.4	104.9	-30.5
	50	60.3	65.4	- 5.1
NSN	0	127.9	131.4	- 3.5
	5	113.6	137.6	-24.0
	8	128.1	132.8	- 4.7
	30	66.9	98.8	-31.9
NSA	0	135.9	126.0	9.9
	1.75	130.2	124.6	5.6
	3.5	114.2	130.3	-16.1
	8	136.9	90.1	46.8
NSO	0	141.4	133.9	7.5
	1.5	125.5	137.4	-11.9
	3	119.1	97.9	21.2
	6	81.4	83.8	- 2.4
Total		1803.7	1860.9	-57.2
Mean		112.7	116.3	- 3.58

$n = 16$ $s = 19.77$ $s_{\bar{x}} = 4.94$ $t = 0.724$ $P \geq 0.4$

Each pair of mean heights was obtained from 32 seedlings grown in the same dish, 16 of which had two roots excised.

roots. The radical emerges as a primary root soon accompanied by two lateral seminal roots. These are in turn followed by another pair laterally disposed, and then finally by two more. In the experiments recorded here, routinely, two roots were excised. These usually consisted of the first two laterals, or, less frequently, the primary root and one lateral. No attempt was made to distinguish between these roots in the cytological examinations which followed.

The squash preparations of the individual root tips were coded before scoring and the members of a pair were not scored consecutively--indeed, in most instances several days intervened. Inspection of the data from a number of paired estimates of per cent normal metaphases (Table 4) shows typical results. The difference in per cent normal metaphase estimates for the two roots seldom exceeded 10%. Additional comparisons of paired estimates can be made for the data illustrated in Figures 5b, 6, and 13.

Effect of very low moisture content

To obtain a very dry state, seeds which had been stored several months over phosphorous pentoxide were pumped several hours and held under vacuum for 24 hours. Before irradiation, the seeds were placed in ampoules which were then evacuated and refilled with either dry air or oxygen at one-half atmosphere positive pressure. This procedure resulted in a moisture content which was determined to be 2.8% (see Table 1).

The frequency distribution of seedling heights for such dry seeds irradiated and stored in dry air or oxygen tends to be bimodal (Figure 1a and 1b) for doses up to about 25kr; beyond this, damage is so great that the population becomes skewed severely toward very low growth.

TABLE 4

COMPARISON OF PER CENT NORMAL METAPHASES AS SCORED ON PAIRS OF ROOTS, EACH PAIR FROM AN INDIVIDUAL SEEDLING

Seeds with 2.8% water content gamma-irradiated and stored as specified.

Dose (kr)	Atmos-phere	Height (mm)	Root A % Normal	Root B % Normal	Differ-ence	Difference x 100 / Mean % Normal
			Pairs With 100 Metaphases Scored Per Root Tip			
2	Air	154	89	82	7	8.2
2	Oxygen	43	47	25	22	61.1
5	Air	128	88	97	9	9.7
5	Air	128	75	70	5	6.9
5	Air	37	81	84	3	3.6
5	Oxygen	166	95	98	3	3.1
5	Oxygen	54	94	94	0	0.0
5	Oxygen	50	93	96	3	3.2
25	Air	120	76	64	12	17.1
25	Air	96	60	57	3	5.1
25	Oxygen	122	64	51	13	22.6
25	Oxygen	48	35	27	8	25.8
				Mean Difference	7.33	
			Pairs With at Least 75 Metaphases Scored Per Root Tip			
1	Air	152	90.0	87.0	3.0	3.4
2	Air	49	51.7	68.5	16.8	28.0
2	Oxygen	142	79.2	68.0	11.2	15.2
2	Oxygen	138	79.0	77.8	0.2	0.3
3	Air	36	7.8	8.0	0.2	2.5
5	Air	138	80.0	85.3	5.3	6.4
5	Air	59	17.0	20.9	3.9	20.5
5	Oxygen	112	56.7	77.0	20.3	30.4
5	Oxygen	26	18.0	23.9	5.9	28.1
9	Air	130	97.4	98.7	1.3	1.3
18	Air	130	89.0	88.7	0.3	0.3
18	Air	60	62.8	54.0	8.8	15.1
36	Air	50	81.4	73.3	8.1	10.5
				Mean Difference	6.56	

At 5 kr the corrected mean height is approximately one-fourth that of the unirradiated control. At this point there is a sharp break in the log per cent height-dose curve (Figure 3). At lower doses there is marked decrease in height as dose increases; beyond this point the decrease is much more gradual.

The height-dose curve of an experiment restricted to the sensitive logarithmic region is presented in Figure 4. It can be observed that an approximate eightfold increase in oxygen tension, during irradiation and storage, over that in the normal atmosphere had only a slight sensitizing effect upon seedling height reduction.

Practically no seedlings from the controls in any of the irradiation experiments had metaphase abnormalities, but, as can be seen in Figures 1 and 2, there is a wide range in the control seedling heights, hence, there is no correlation between seedling height and per cent normal metaphases for the unirradiated treatments. Despite this, a high positive correlation on an individual basis does exist between these two criteria for the seeds which were gamma-irradiated with 1, 2, 3, 4 or 5 kr. Statistical analysis gave a value for the correlation coefficient, $r = 0.885$ with a probability that the data are not correlated, $P < 0.001$. A distribution plot for seedling height versus per cent normal metaphases for seeds irradiated and stored in air is given in Figure 5a. Each point represents the mean value for the independent estimates of per cent normal metaphases from each of two root tips from a seedling of the height indicated. The regression line for seedling height on per cent normal metaphases is shown.

In Figure 5b, similar data for seeds irradiated and stored in oxygen at one-half atmosphere positive pressure are presented. Here

FIGURE 3

PLOT OF LOG MEAN SEEDLING HEIGHT VERSUS GAMMA-RAY DOSE FOR BARLEY SEEDS WITH 2.8% WATER CONTENT; IRRADIATED AT HIGH DOSES AND STORED 90 DAYS POST-IRRADIATION

Irradiation and storage was either in dry air or in oxygen at one-half atmosphere positive pressure. Doses were 0, 9, 18, 36 kr Co^{60} gamma-irradiation at 4.25 kr/min with approximately 100 seeds per treatment. Seedlings were measured after eight days growth.

The mean height for each treatment, minus the elongation height (16 mm), is plotted as log per cent of the corrected, pooled control height, 98.9 mm.

The data indicate no appreciable difference between the two treatments at these doses. Note the sharp break in the curve which occurs at or below 5 kr. With this dose damage is so severe that further increments of dose have only slight additional effect.

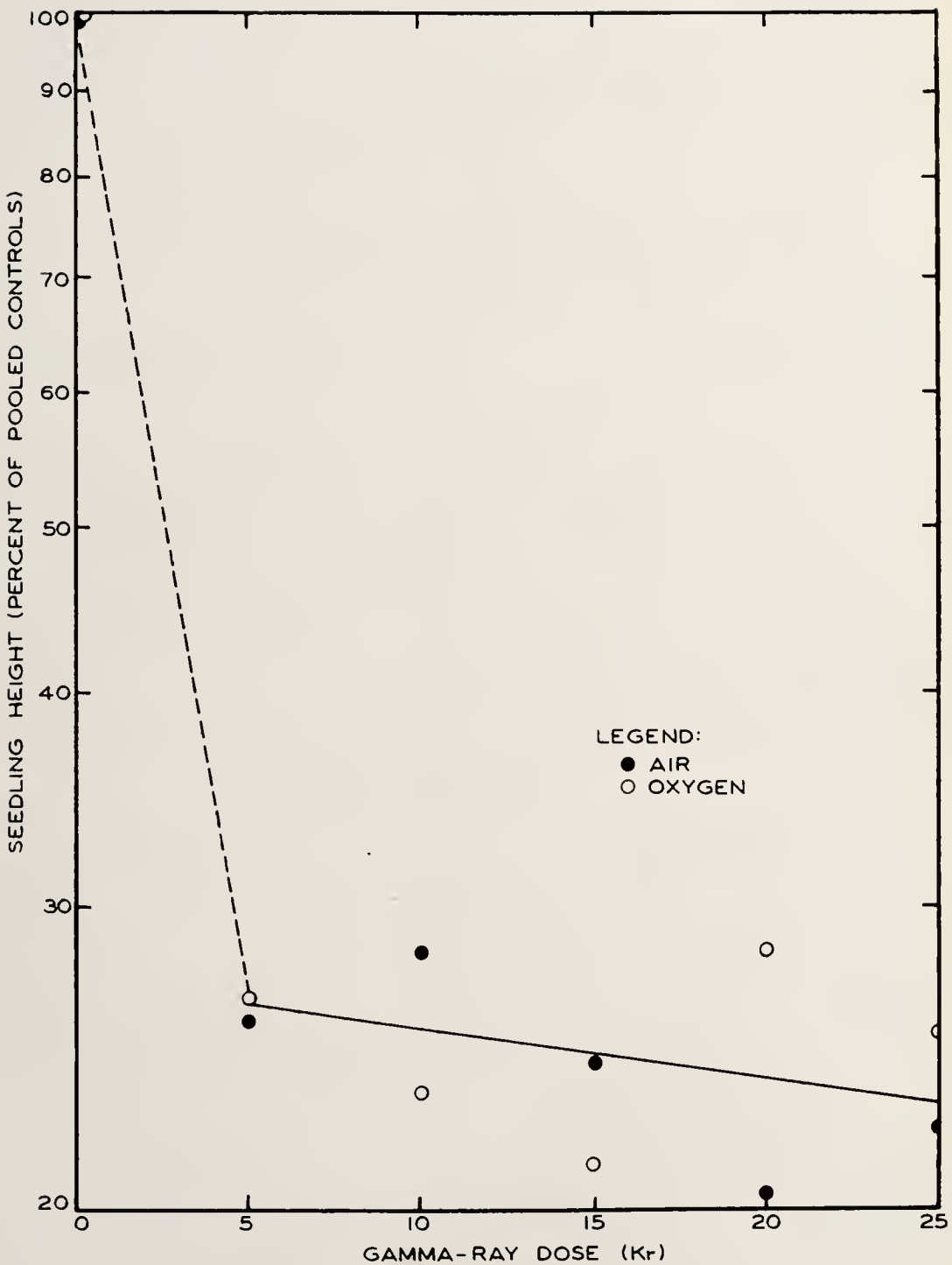

SEEDLING HEIGHT (PERCENT OF POOLED CONTROLS)
100
90
80
70
60
50
40
30
20
0
5
10
15
20
25
GAMMA-RAY DOSE (Kr)
LEGEND:
AIR
OXYGEN

FIGURE 4

PLOT OF MEAN SEEDLING HEIGHT VERSUS GAMMA-RAY DOSE FOR BARLEY SEEDS WITH 2.8% WATER CONTENT; IRRADIATED AT LOW DOSES AND STORED 20 DAYS POST-IRRADIATION

Irradiation and storage were either in dry air or in oxygen at one-half atmosphere positive pressure. Doses were 0, 1, 2, 3, 4, and 5 kr at 4.05 kr/min with 100 seeds per treatment. Seedlings were measured after eight days growth.

The mean height for each treatment, minus the elongation height (16 mm), is plotted as per cent of the corrected specific control height, 92.1 mm for air and 87.9 mm for oxygen.

The data suggest slight differences between the two atmosphere treatments at the doses used. The points for oxygen in all cases but one (2 kr) fall below those for air even though they are relative to a slightly smaller control value. The general sigmoidal form of the curve as drawn by inspection is typical of response over this dose range.

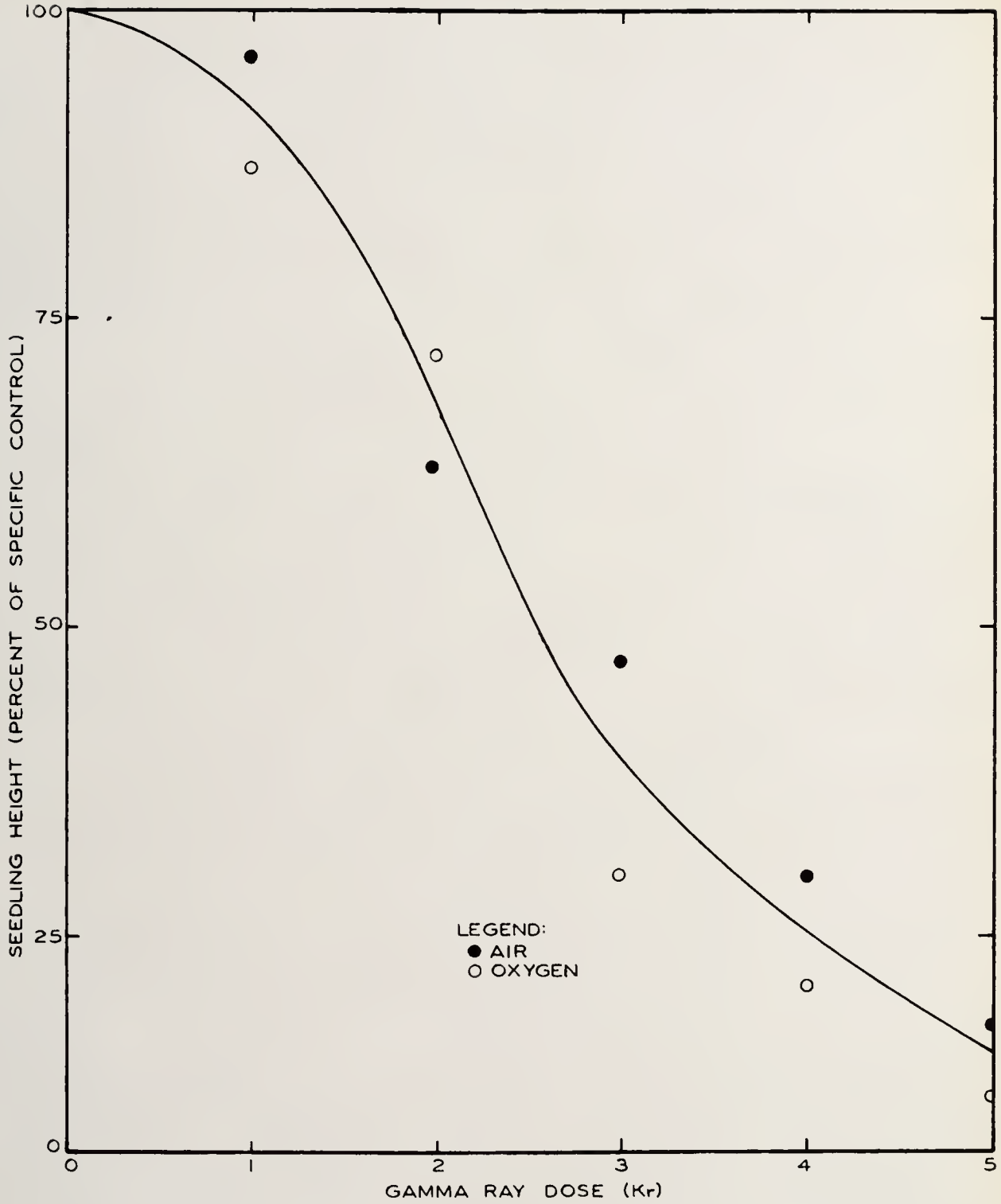

100
75
50
25
0
SEEDLING HEIGHT (PERCENT OF SPECIFIC CONTROL)
0
1
2
3
4
5
GAMMA RAY DOSE (Kr)
LEGEND:
AIR
OXYGEN

FIGURE 5a

REGRESSION OF SEEDLING HEIGHT ON PER CENT NORMAL METAPHASES FOR SEEDS WITH 2.8% WATER CONTENT; GAMMA-IRRADIATED AT LOW DOSES AND STORED 20 DAYS IN DRY AIR

Treatment mean heights and other data for this experiment are given in Figure 4.

Two root tips were excised from each seedling during the second day of growth and analyzed separately for per cent normal metaphases. An average of 50 metaphases was scored per slide resulting in a mean difference of approximately 7% normal metaphases between the members of a pair. The mean value of each pair (in a few cases, the value for a single root tip where the partner was unanalyzable) is plotted as a point on the distribution. The regression equation for seedling height on per cent normal metaphases is $\hat{Y} = 24.1 + 1.153X$ and the standard error of estimate, $s_e = 17.3$ mm. The controls are not included in this regression since in all those examined there were no aberrations regardless of height, even though most of them were shorter than the mean height for controls.

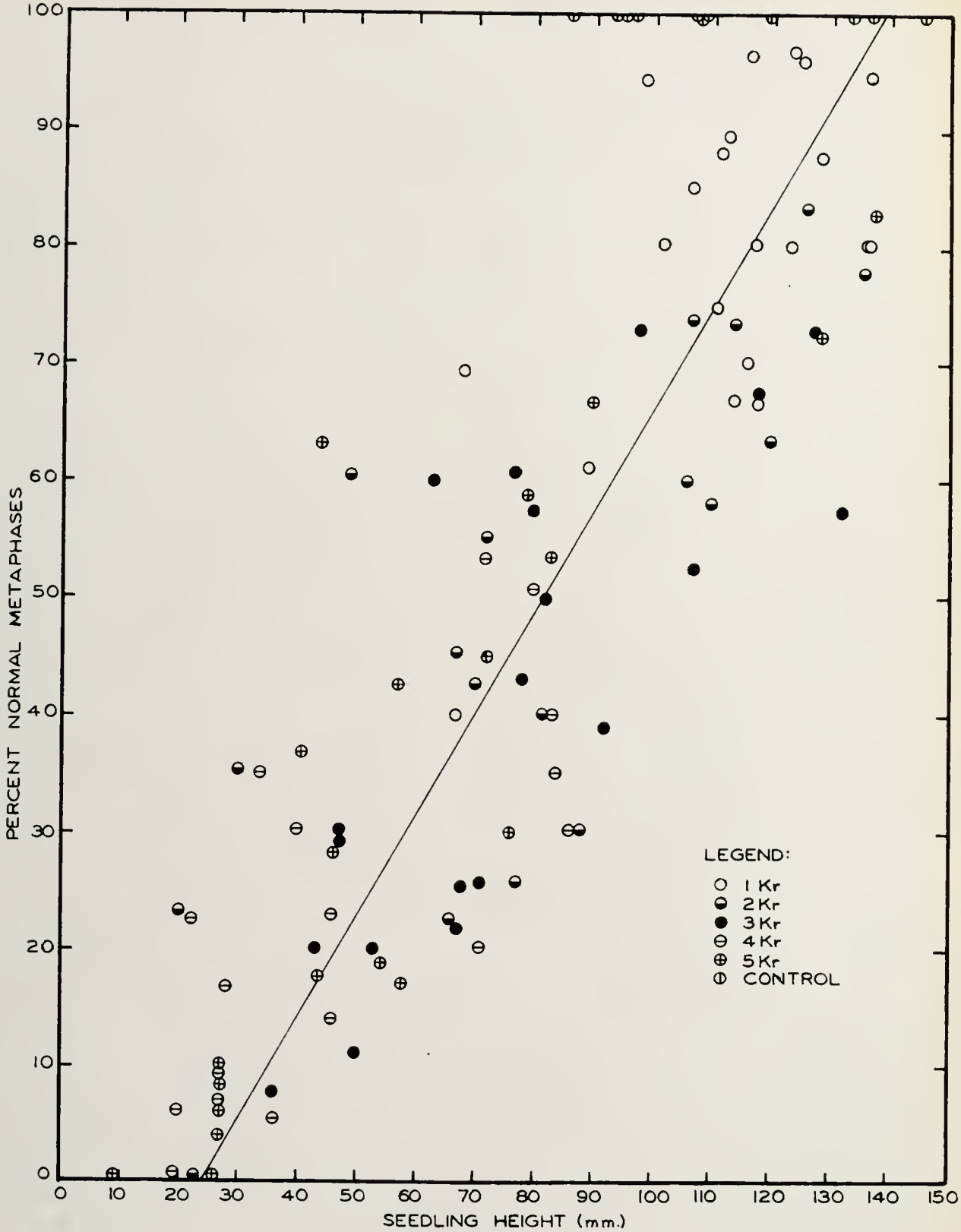
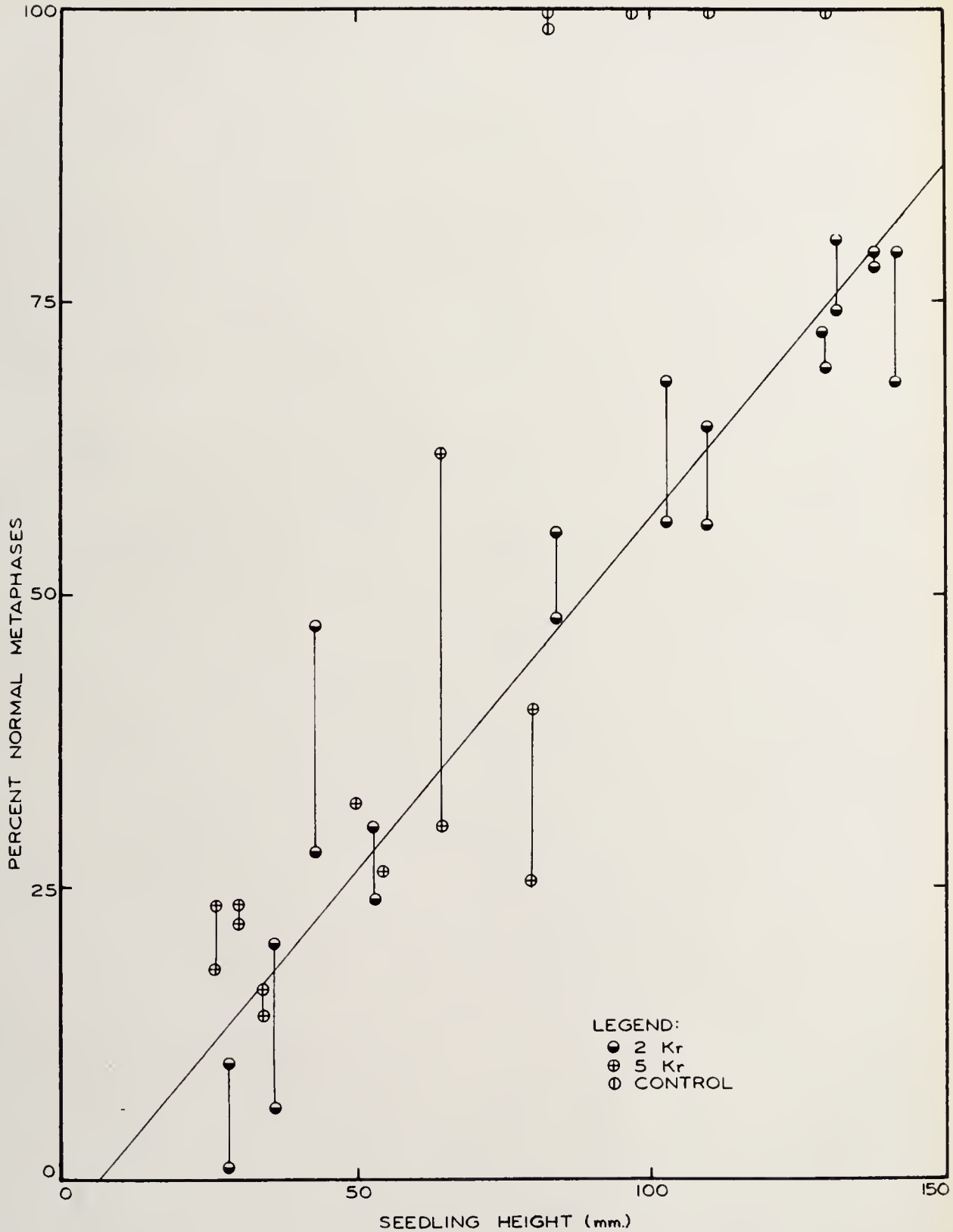

PERCENT NORMAL METAPHASES
SEEDLING HEIGHT (mm.)
LEGEND:
1 Kr
2 Kr
3 Kr
4 Kr
5 Kr
CONTROL

FIGURE 5b

REGRESSION OF SEEDLING HEIGHT ON PER CENT NORMAL METAPHASES FOR SEEDS WITH 2.8% WATER CONTENT; GAMMA-IRRADIATED AT LOW DOSES AND STORED 20 DAYS IN OXYGEN AT ONE-HALF ATMOSPHERE POSITIVE PRESSURE

Treatment mean heights and other data for this experiment are given in Figure 4 and general procedural details accompany Figure 5a.

Each root tip was analyzed separately. The pair observations are plotted separately and connected by vertical lines. Observations were made only on 0, 2 and 5 kr doses as indicated by the appropriate symbols on the figure. With the exception of two seedlings all values are based on a minimum of 50 analyzed metaphases. The regression equation for seedling height on per cent normal metaphases is $\hat{Y} = 4.32 + 1.683X$ and the standard error of estimate, $s_e = 29.0$ mm. As in 5a the controls are not included in this regression. In the shorter-than-average controls examined a single abnormal metaphase was observed.


100
75
50
25
0
0
50
100
150
PERCENT NORMAL METAPHASES
SEEDLING HEIGHT (mm.)
LEGEND:
2 Kr
5 Kr
CONTROL

the value for each root tip is plotted, the pairs being connected by vertical lines. Data was obtained only for the 2 and 5 kr doses. For these data $r = 0.955$ with probability $P < 0.001$ that this is a chance association.

These data further indicate that within the dose range of this experiment the length attained by individual seedlings is a function of per cent normal cells, irrespective of the dose received. Similar results were obtained in other experiments with doses up to 36 kr, though fewer observations were made at these higher levels. Figure 6 shows the distribution of data from one such experiment. Here, the value for each root tip is indicated and the pairs are connected by vertical lines as in Figure 5b. Data for air and oxygen treatments are combined in this analysis of seeds given 5, 15, or 25 kr gamma irradiation. The correlation coefficient, $r = 0.913$ with $P < 0.001$.

A logarithmic plot of per cent corrected specific mean control height, and of per cent normal metaphases versus dose for the experiment illustrated in Figure 4 is present in Figure 7, and a summary of the data is given in Table 5. The curve for per cent normal metaphases was determined from subsamples of those individuals which were examined cytologically. These subsamples were selected to have mean heights equal to those of the whole population at each dose level. Since, for every dose level the total cytological sample had a higher mean height than that of the whole treatment, for reasons previously stated, the representative subsample was obtained by exclusion of a sufficient number of the highest plants to reduce the mean height to that of the whole treatment. This procedure was used to prevent subjective bias in selection.

FIGURE 6

REGRESSION OF SEEDLING HEIGHT ON PER CENT NORMAL METAPHASES FOR SEEDS WITH 2.8% WATER CONTENT; GAMMA-IRRADIATED AT HIGH DOSES AND STORED 90 DAYS IN DRY AIR OR IN OXYGEN AT ONE-HALF ATMOSPHERE POSITIVE PRESSURE

Treatment mean heights and other data for this experiment are given in Figure 3; procedural details are the same as those accompanying Figure 5b.

No distinction is made, here, between the air and oxygen treatment. Only root tips of seedlings from 5, 15 and 25 kr doses were analyzed in this experiment. The three seedlings represented by the points in the upper left of the figure were excluded from the calculations since their growth was reduced by causes extraneous to this experiment (fungal infection).

The regression equation for seedling height on per cent normal metaphases is $\hat{Y} = -10.7 + 1.827X$ and the standard error of estimate, $s_e = 33.4$ mm. Note that the horizontal axis has its origin at 20 mm.

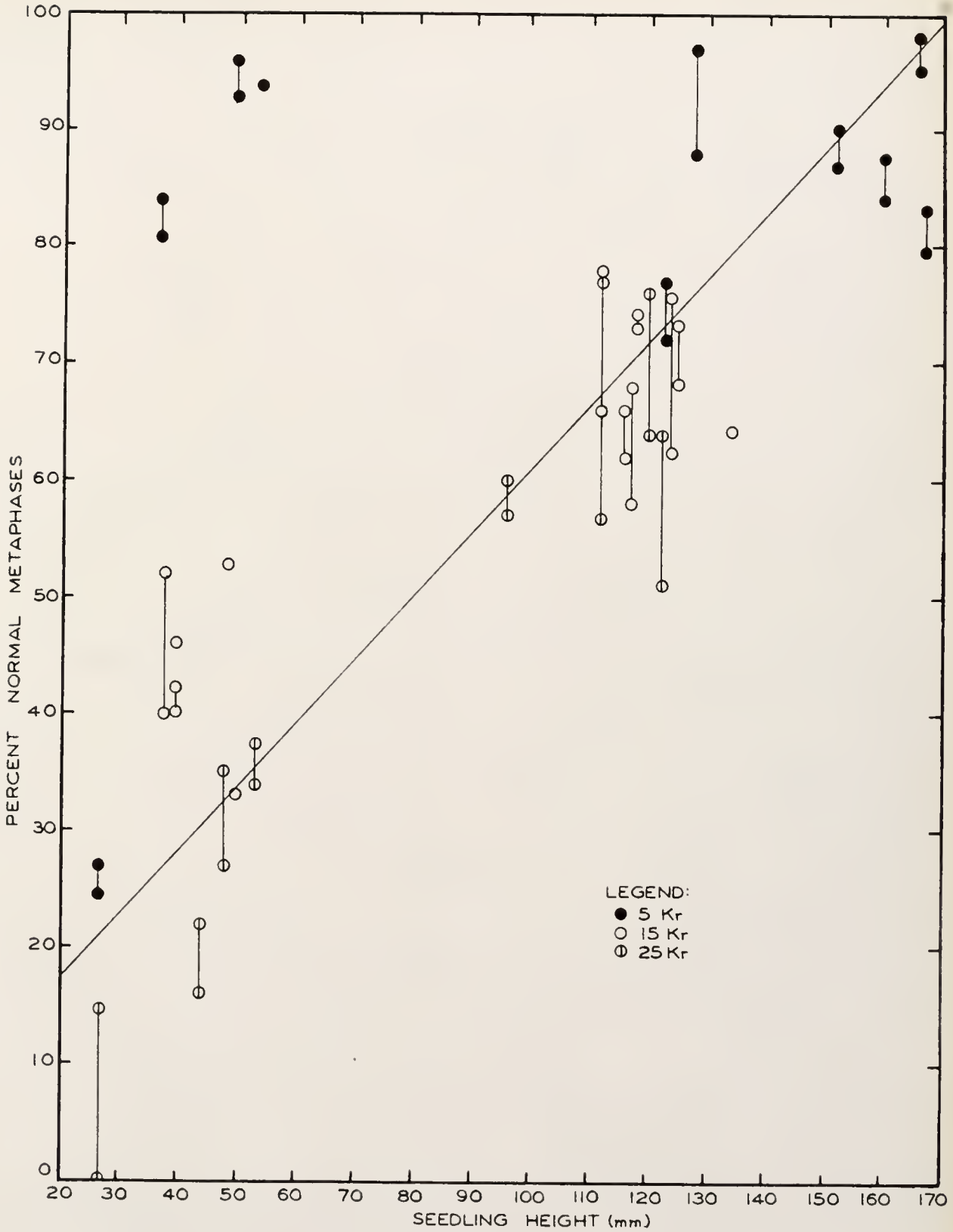

PERCENT NORMAL METAPHASES
SEEDLING HEIGHT (mm)
LEGEND:
5 Kr
15 Kr
25 Kr

FIGURE 7

PLOT OF LOG MEAN PER CENT NORMAL METAPHASES AND LOG MEAN SEEDLING HEIGHT AT 8 DAYS VERSUS DOSE FOR SEEDS WITH 2.8% WATER CONTENT; GAMMA-IRRADIATED AND STORED 20 DAYS IN DRY AIR

The cytological data are a portion of that presented in different form in Figure 5a. The method for selection of these data is discussed in the text and the data are summarized in Table 5.

The treatment mean heights for the seedlings contributing to the cytological data are identical with those for the total population which are plotted here. Thus direct comparisons can be made between the two curves.

The regression equation for log seedling height on dose is $\log \hat{Y} = 150.7 - 1.517X$ and that for per cent normal metaphases on dose is $\log \hat{Y} = 114.8 - 1.551X$.

The HD_{50} (that dose which reduces the height to one-half that of control) is 2.65 kr; the CD_{50} (that dose which reduces per cent normal metaphases to one-half) is 1.90 kr.

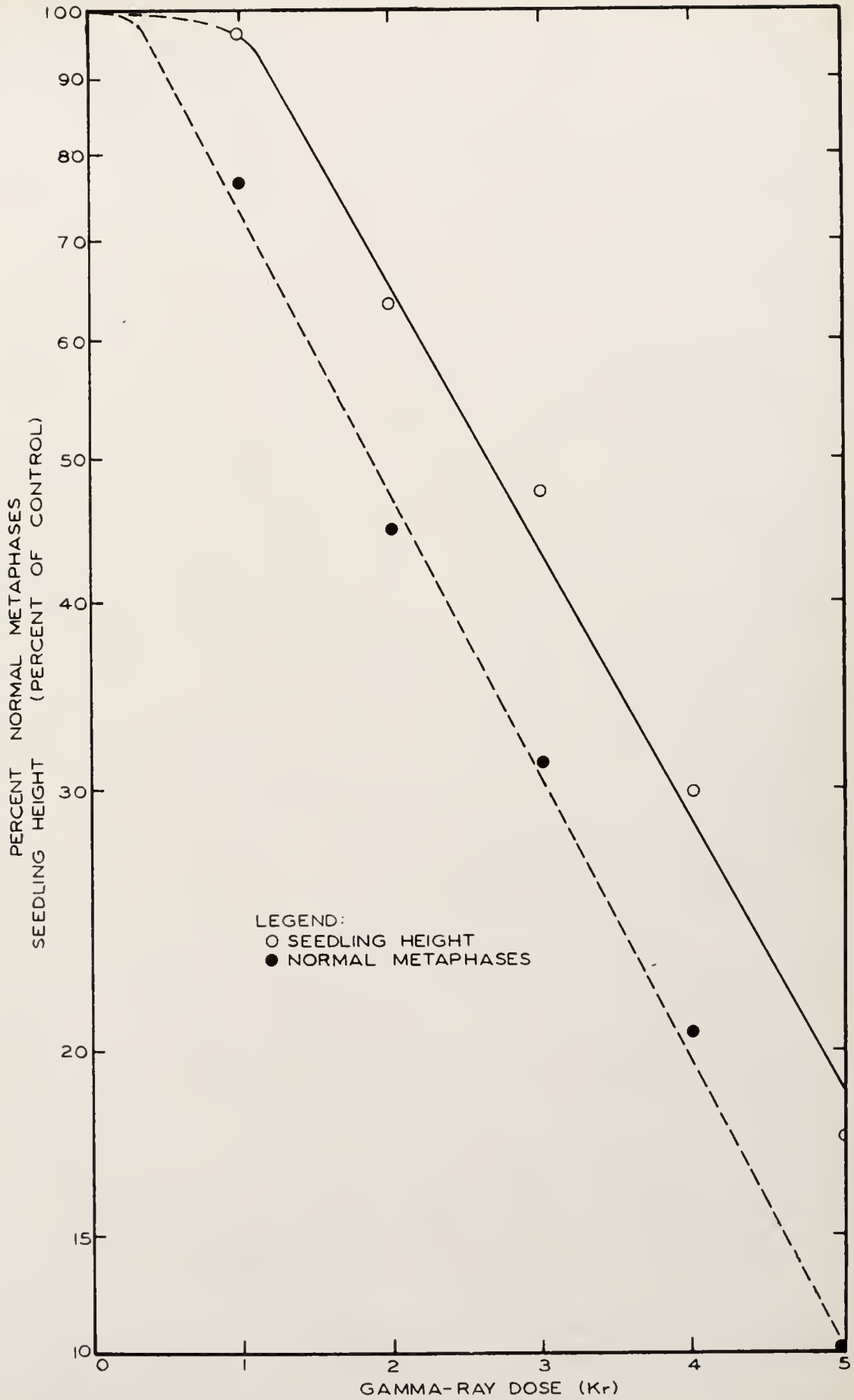

PERCENT NORMAL METAPHASES
SEEDLING HEIGHT (PERCENT OF CONTROL)
100
90
80
70
60
50
40
30
20
15
10
0
1
2
3
4
5
GAMMA-RAY DOSE (Kr)
LEGEND:
O SEEDLING HEIGHT
● NORMAL METAPHASES

TABLE 5

MEAN SEEDLING HEIGHT FOR TREATMENTS AT DIFFERENT DOSES AND FOR SAMPLES EXAMINED CYTOLOGICALLY FOR PER CENT NORMAL METAPHASES

Seeds with 2.8% water content gamma-irradiated in air and stored 20 days in dry air post-irradiation. The representative cytological sub-samples were selected to have mean heights corresponding to those of the entire treatment. Seedlings measured at 8 days.

	Gamma-Ray Dose (kr)					
	0	1	2	3	4	5
Entire Treatment						
Number	97	97	84	82	81	83
Mean Height (mm) (corrected)*	92.1	88.6	58.3	43.7	27.4	16.1
% Control Height (mm) (corrected)*	100	96.2	63.3	47.4	29.8	17.5
Entire Cytological Sample						
Number	12	21	19	20	17	19
Mean Height (mm) (corrected)*	95.6	96.6	69.1	63.9	32.4	42.4
% Normal Meta-phases	100	91.4	50.5	41.2	21.9	31.4
Representative Cytological Sub-Sample						
Number		15	16	13	15	10
Mean Height (mm) (corrected)*		89.0	59.1	44.0	27.5	16.9
% Normal Meta-phases		76.1	44.7	31.2	20.5	12.5

*Mean height (corrected) equals actual mean height minus 16 mm, height due to elongation.

The fact that the curve for cytological effects falls below that for gross effects indicates that the cytological effects are more radiosensitive. There is approximately 700 r difference for equal response of cytological and gross effects relative to controls throughout the range tested. The 50% level of effect for height reduction (HD_{50}), after correction for cell elongation (see methods section) was approximately 2.7 kr in this experiment, while that for chromosomal damage (CD_{50}) was 1.9 kr, giving a relative sensitivity of 1.4 for these two criteria of radiation damage.

The regression lines do not intercept the response axis at zero dose but instead indicate the presence of a threshold around 300 r for chromosomes and 980 r for leaf length. This phenomenon was not investigated further in these experiments, but it is a common observation in many radiation experiments, i. e. a "multi-hit" response. It is interpreted as implying that several independent unitary events of the type causing damage are required before damage is made manifest. It is known, furthermore, that very low doses may actually stimulate growth (e. g. Suess, 1961).

The results of these experiments with very dry seeds irradiated and stored in dry air or oxygen at room temperature suggest that the heterogeneous growth response (Figure 1) is a consequence of chromosomal damage which differs greatly in extent among the individual irradiated seeds. The cause for this difference among seeds receiving the same treatment is not known.

Effect of higher moisture contents

For these experiments seeds were stored at a wide range of different humidities until their moisture content was stabilized. Samples

were withdrawn, placed in glass tubes fitted with rubber stoppers, irradiated in air and returned to their respective humidities for post-irradiation storage.

Height-dose regression lines for the composite data of three experiments are given in Figure 8. The results indicate the inverse relationship between moisture content of the seeds and radiation damage to growth which occurs over this range of humidities. There is scatter in the data, much of which results from difficulty in obtaining the same degree of response from experiment to experiment. This difficulty is encountered frequently in this material and will be referred to in the discussion.

In Figure 9 the interpolated values for doses giving a corrected mean seedling height 50% that of the related control (HD_{50}) are plotted against water content. For comparison similar data from Ehrenberg (1955b) are included. There is indication of a maximum effect around 13% water with a decrease at higher as well as at lower values, but this particular point was studied in only one experiment and not confirmed.

Cytological observations were made on individuals from the various treatments within one such experiment. Particular effort was made to collect data from the dose at each moisture level in the midrange of the "log phase" of response to dose, i. e. 40% of corrected specific control mean height. This was done in order to determine whether reduction in seedling height is directly related to observable chromosomal damage over a wide range of seed water content. The results are presented graphically in Figure 10 and are summarized in Table 6. The regressions of seedling height upon per cent normal metaphases as

FIGURE 8

REGRESSION OF LOG MEAN SEEDLING HEIGHT ON GAMMA-RAY DOSE FOR SEEDS WITH DIFFERENT WATER CONTENTS; COMPOSITE DATA FROM THREE EXPERIMENTS

All seeds were stored over appropriate desiccant or solution as specified in Table 1 for several months prior to use, with the exception of the wettest treatment which was stored for only several weeks to prevent impaired germination. Following irradiation in air all treatments were returned to their respective desiccators for post-irradiation storage.

	Experiment		
	a	b	c
Days stored post-irradiation	8	6	38
Days growth before measurement	9	8	9

Points from individual experiments are indicated by appropriate letter. Data at 2.8% water from Figure 7 are included.

Straight lines were fitted to the data by linear regression and that dose which gives 50% height reduction relative to specific control (HD_{50}) was determined.

Per Cent Water	Regression Equation	HD_{50}	Relative Sensitivity
2.8	$\hat{Y} = 150.7 - 1.517X$	2.65	15.1
3.8	$\hat{Y} = 86.9 - 1.263X$	2.6	15.4
6.7	$\hat{Y} = 120.5 - 1.252X$	4.0	10.0
7.3	$\hat{Y} = 85.9 - 1.119X$	5.0	8.0
9.2	$\hat{Y} = 110.0 - 1.113X$	7.3	5.5
10.1	$\hat{Y} = 133.7 - 1.079X$	13	3.1
12.7	$\hat{Y} = 124.7 - 1.023X$	40	1
15.5	$\hat{Y} = 225.7 - 1.063X$	25	1.6

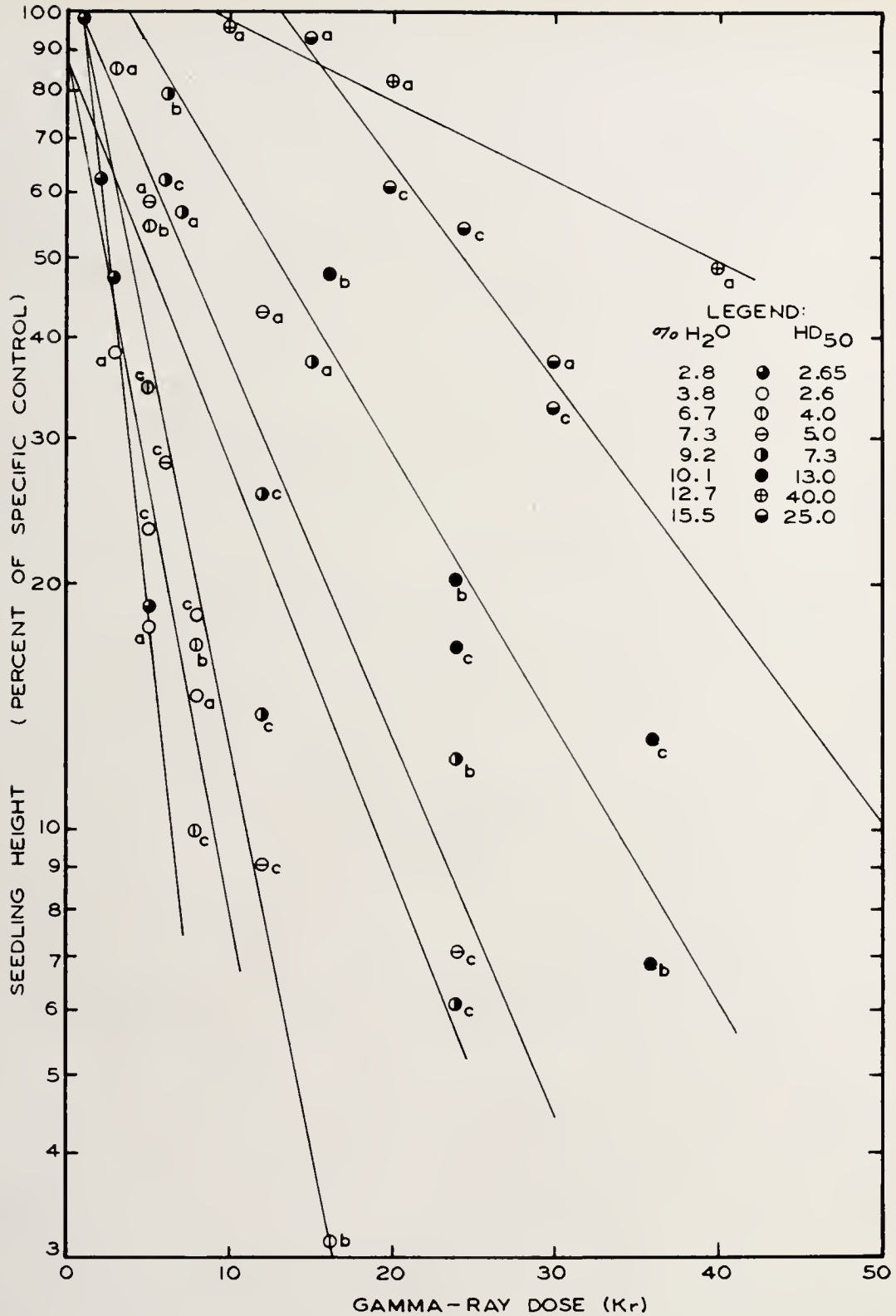

SEEDLING HEIGHT (PERCENT OF SPECIFIC CONTROL)
GAMMA-RAY DOSE (Kr)
LEGEND:
% H2O
HD50
2.8 2.65
3.8 2.6
6.7 4.0
7.3 5.0
9.2 7.3
10.1 13.0
12.7 40.0
15.5 25.0

FIGURE 9

RELATIVE SENSITIVITY OF SEEDS OF DIFFERENT WATER CONTENT GAMMA-IRRADIATED AND STORED IN AIR, NITROGEN OR OXYGEN

The HD_{50} (that dose reducing seedling growth to 50% that of unirradiated controls) values obtained from the data in Figure 8 are plotted against seed water content (heavy line). For comparison the data from Ehrenberg (1955b) for x-rayed barley seed treatments in nitrogen and oxygen are included.

The point for 12.7% water content was determined from a single experiment and not confirmed.

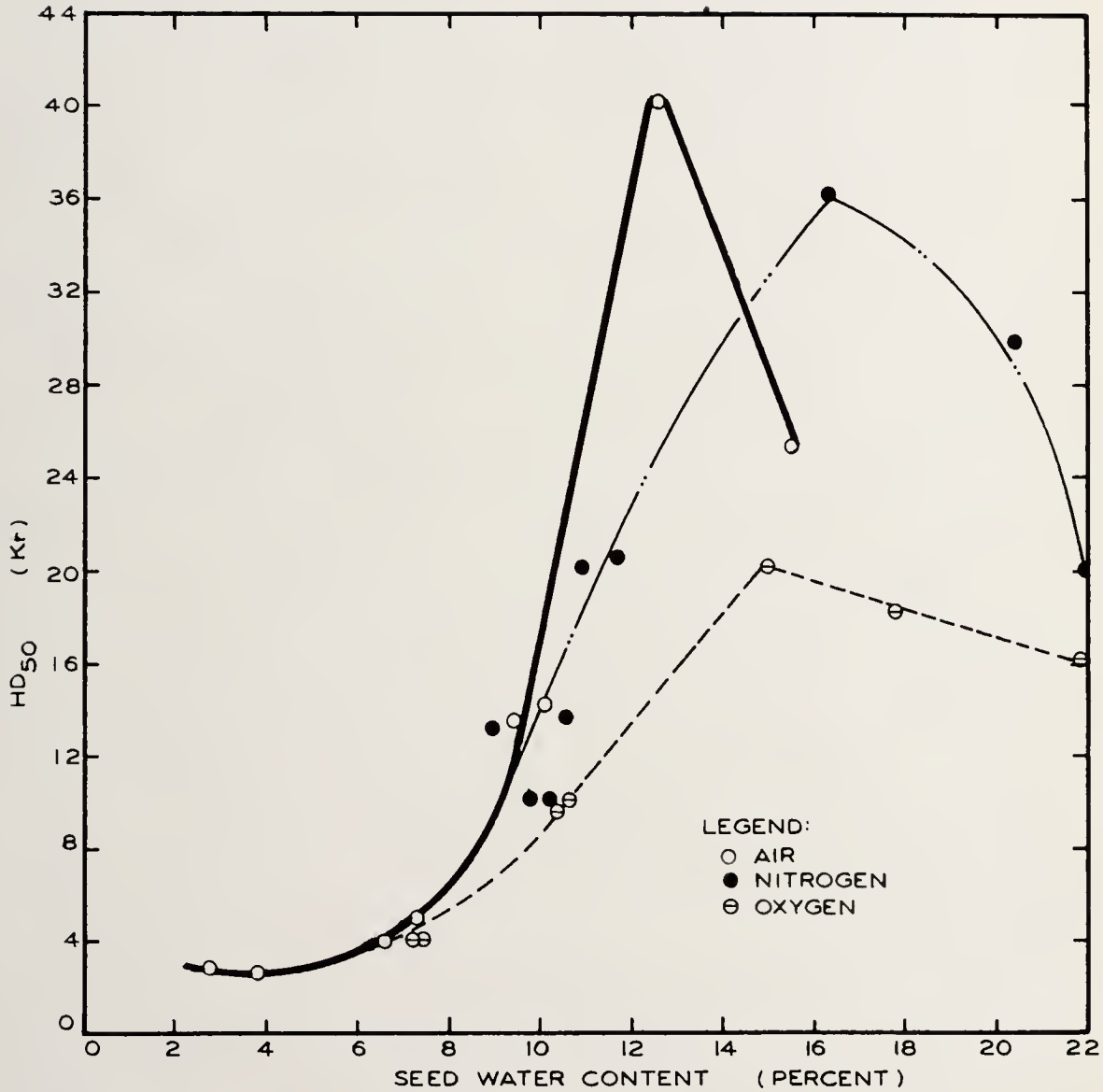

44
40
36
32
28
24
20
16
12
8
4
0
HD_{50} (Kr)
0
2
4
6
8
10
12
14
16
18
20
22
SEED WATER CONTENT (PERCENT)
LEGEND:
AIR
NITROGEN
OXYGEN

FIGURE 10

REGRESSION OF SEEDLING HEIGHT ON PER CENT NORMAL METAPHASES FOR SEEDS OF DIFFERENT WATER CONTENT IRRADIATED AND STORED IN AIR

Details for this experiment are those of Experiment a, Figure 8. The cytological data and statistical computations are presented in Table 6. The cytological analysis presented here was made on seedlings from that dose at each moisture level closest to 40% control height. The actual values and the regression equations from Table 6 are summarized here.

Seed Moisture Content, in Per Cent	Dose (kr)	Mean Height as Per Cent of Control	Regression Equation
3.8	3	38.4	$\hat{Y} = 7.37 + 1.178X$
6.7	8	40.6	$\hat{Y} = -10.98 + 1.538X$
9.2	15	37.7	$\hat{Y} = 1.20 + 1.681X$
12.7	40	48.5	$\hat{Y} = 9.69 + 1.702X$
15.5	30	37.1	$\hat{Y} = 5.05 + 1.131X$

The analysis indicates that the regression of seedling height on cytological damage is not affected by differences in water content.

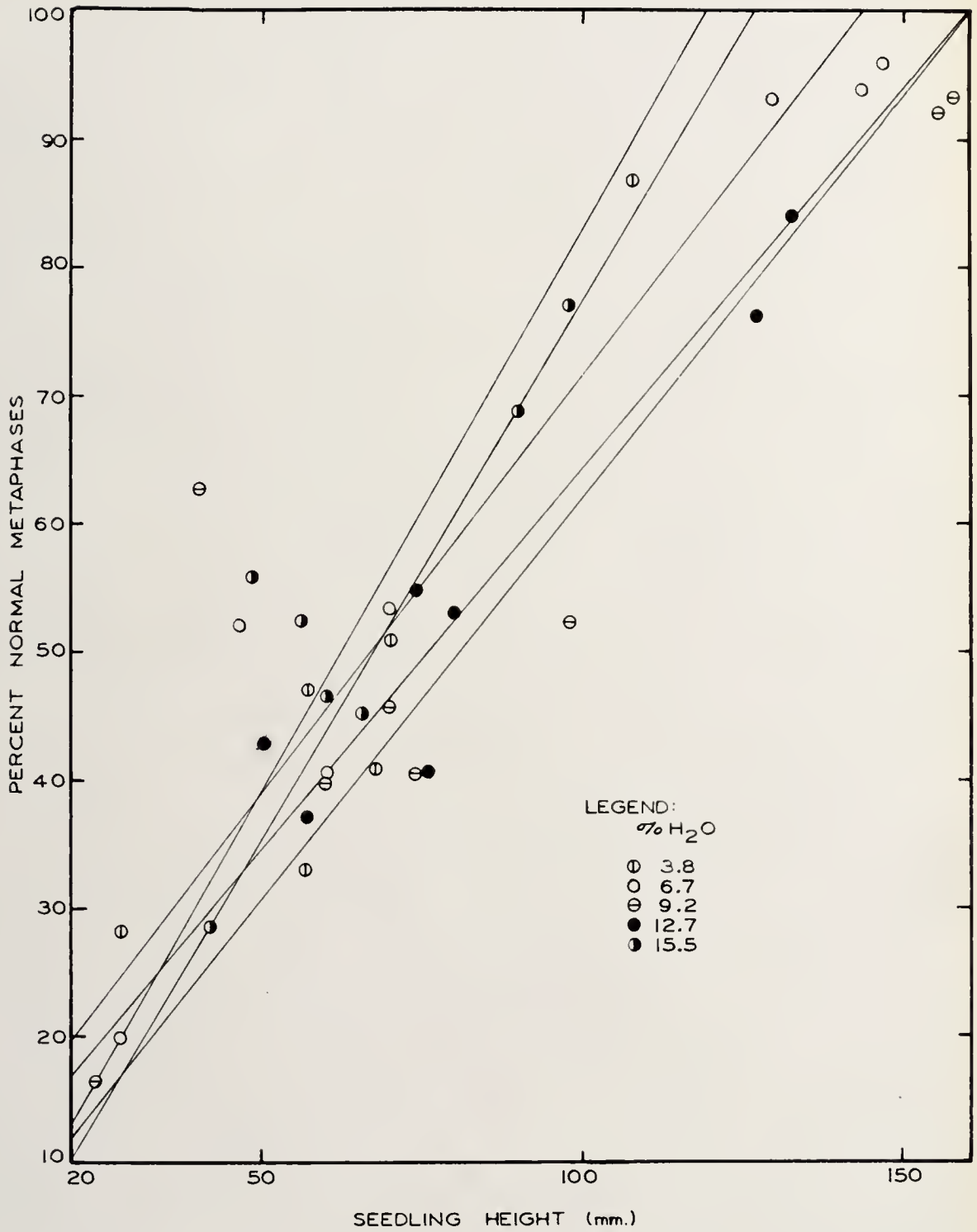

PERCENT NORMAL METAPHASES
SEEDLING HEIGHT (mm.)
100
90
80
70
60
50
40
30
20
10
20
50
100
150
LEGEND:
% H2O
3.8
6.7
9.2
12.7
15.5

TABLE 6

SEEDLING HEIGHT AND PER CENT NORMAL METAPHASES FOR SEEDS OF DIFFERENT WATER CONTENT, WITH SUMMARY OF CORRELATION ANALYSIS

Seeds gamma-irradiated and stored at constant water content in air 8 days post-irradiation. Seedlings measured at 9 days.

Seed Water Content (%)	3.8		6.7		9.2		12.7		15.5	
Dose (kr)	3		8		15		40		30	
	Height (mm)	Normal (%)	Height (mm)	Normal (%)	Height (mm)	Normal (%)	Height (mm)	Normal (%)	Height (mm)	Normal (%)
	108	86.6	147	96.0	158	93.0	132	84.0	98	77.0
	70	50.8	144	94.0	156	92.0	127	76.0	90	69.0
	63	40.8	120	93.5	98	52.5	80	53.4	66	45.0
	57	47.3	70	53.3	74	40.3	76	40.8	60	46.7
	57	33.3	60	40.8	70	45.9	74	55.0	56	52.5
	28	28.3	46	52.0	60	40.3	57	37.5	48	56.0
			28	20.0	40	65.0	50	43.0	42	29.0
					24	11.7				
Sum	383	287.3	615	449.6	680	440.7	596	389.7	460	375.2
Mean	63.8	47.9	87.9	64.2	85.0	55.1	85.1	55.7	65.7	53.6
Treatment Mean	38.4		40.6		37.7		48.5		37.1	

Regressions

Height on % Normal: $\hat{Y} = 7.37 + 1.178X$ $-10.97 + 1.54X$ $-0.33 + 1.549X$ $-9.69 + 1.702X$ $5.05 + 1.131X$

% Normal on Height: $\hat{X} = -0.2 + 0.754Y$ $11.27 + 0.602Y$ $15.39 + 0.475Y$ $10.61 + 0.530Y$ $10.77 + 0.652Y$

Correlation Coefficient

$r_1 = 0.9423$ $r_2 = 0.9624$ $r_3 = 0.8601$ $r_4 = 0.9497$ $r_5 = 0.8587$

$z_1 = 1.758$ $z_2 = 1.9781$ $z_3 = 1.293$ $z_4 = 1.829$ $z_5 = 1.287$

$s_{\bar{d}_{2,3}} = 0.6708$ $t = 1.021$ d.f. $= \infty$ $P > 0.3$ All other comparisons have greater values of P, hence, the correlations do not differ significantly.

determined for each moisture level do not differ significantly. The regression lines for 3.8 and 15.5% water content appear somewhat divergent from the others, probably because the seedlings at these two moisture levels displayed impaired growth in the unirradiated controls when compared to those of the other moisture levels. Such reduced vigor can reasonably be expected in the irradiated individuals as well.

Supplementary data from other treatments within the same experiment as well as the general experience of the writer confirm the direct relationship of reduction in seedling height and observable chromosomal damage at the individual level within the wide range of water contents and consequent radiosensitivities which were examined.

Effects of post-irradiation storage and oxygen

The seeds used in these experiments were stored for several months over phosphorus pentoxide prior to use. Their initial water content was not measured for every experiment but was assumed to be about 3.8% as previously determined for these conditions. After vigorous pumping and flushing (see methods section), all seed samples were sealed in glass ampoules in an anoxic atmosphere of nitrogen for at least two days before irradiation.

After irradiation some seed samples were soaked immediately (NI) within 30 seconds post-irradiation. Other ampoules were opened and placed, within 45 seconds, in a pressure bomb which was held at 200 pounds over atmospheric in dry oxygen (NSO) giving an oxygen concentration approximately 65 times that of air. Still others were opened to air via a drying column packed with silica gel and then transferred to storage in air over phosphorus pentoxide (NSA). Finally, one group was left sealed in the atmosphere of nitrogen in which it was irradiated (NSN). Post-irradiation storage was four to five days.

In a preliminary experiment of this nature using helium as an anoxic atmosphere, other workers in this laboratory (Gennaro and Harrer, unpublished) obtained the growth and cytological response to dose shown in Figure 11. (Coding of the treatments is the same as that given above except that He was used instead of N.) The cytological data were collected from root tips of an unmeasured subsample. A good correspondence was obtained between damage to the chromosomes and reduced height. There was considerable scatter in the data; and it is interesting to note that especially for the least sensitive treatment (HeI) the cytological response was more precise than that of seedling height, although these observations were based on many fewer individuals. Their use of oxygen at 1750 pounds pressure resulted in chromosomal aberrations in 17% of the metaphases examined in unirradiated controls.

In a recent experiment (Figures 12 and 13) individuals were examined cytologically from that dose level, for each type of post-irradiation treatment, which resulted in a mean seedling height between 40 and 50% of control height. Correlations between height and per cent normal metaphases for these groups of individuals did not differ significantly (Table 7) despite doses as disparate as 6 and 50 kr. Agreement would probably have been even greater had the range of heights sampled been more nearly the same in all treatments.

General cytological observations

All observations were made on root tip cells arrested by colchicine at metaphase of the first division cycle upon sprouting of the seeds. The criterion employed in these experiments for scoring chromosomal effects was the presence or absence of any detectable chromosomal aberration. Records of type and number of aberrations with a given cell were made only on cells of unusual interest.

FIGURE 11

PLOT OF LOG MEAN PER CENT NORMAL METAPHASES AND LOG MEAN SEEDLING HEIGHT VERSUS DOSE FOR BARLEY SEEDS GAMMA-IRRADIATED IN HELIUM AND STORED AT DIFFERENT OXYGEN TENSIONS

Doses were 0, 0.67, 1.33, 2.65, 5.3, 10.6, 21.2 and 31.8 kr at 5.3 kr/min. Seedlings were measured after seven days growth.

The treatments were as follows: The seeds had a water content of approximately 3.8%; all seed ampoules were evacuated and flushed repeatedly and then stored at a slight positive pressure of helium for one day prior to irradiation.

HeI — immediately after irradiation seeds were soaked in anoxic water and germinated.

HeSA — immediately after irradiation seed ampoules were evacuated and dry air admitted for 3 days storage prior to germination.

HeSO — immediately after irradiation seed ampoules were opened and placed in a pressure bomb and stored 3 days in oxygen at 1750 pounds pressure prior to germination. This is approximately 585 times the concentration of oxygen in air.

The doses (in kr) giving 50% reduction for height and for normal metaphases as well as the associated relative sensitivities estimated from this plot are:

	HD_{50}	Relative Sensitivity	CD_{50}	Relative Sensitivity
HeI	50	1	20	1
HeSA	5.3	9.4	3.2	6.2
HeSO	1.7	29.4	1.0	20

This data is from an experiment by Gennaro and Harrer (unpublished) and seedling heights were not corrected for elongation. Such "correction" steepens the slope of the seedling height lines making the comparison with per cent normal even closer.

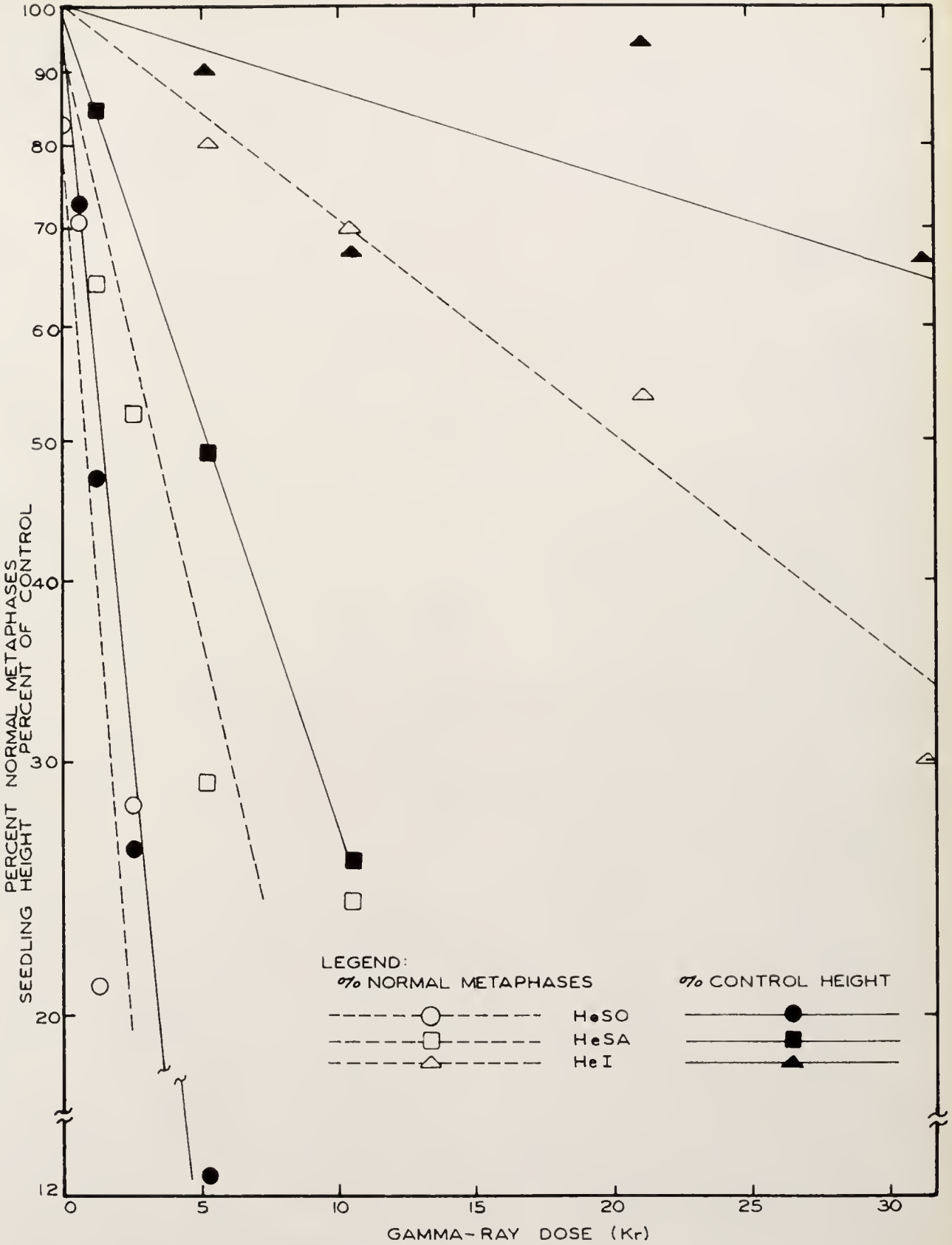

PERCENT NORMAL METAPHASES
PERCENT OF CONTROL
SEEDLING HEIGHT
100
90
80
70
60
50
40
30
20
12
0
5
10
15
20
25
30
GAMMA-RAY DOSE (Kr)
LEGEND:
% NORMAL METAPHASES
% CONTROL HEIGHT
HeSO
HeSA
HeI

FIGURE 12

PLOT OF LOG MEAN SEEDLING HEIGHT VERSUS DOSE FOR BARLEY SEEDS WITH 3.8% WATER CONTENT, GAMMA-IRRADIATED IN NITROGEN AND STORED AT DIFFERENT OXYGEN TENSIONS

Doses were 0, 1.5, 1.75, 2.5, 3, 3.5, 6, 8, 30 and 50 kr at 3.83 kr/min. Seedlings were measured after nine days growth.

The treatments were as follows: Approximately seventy-five seeds were placed in each ampoule; all seed ampoules were evacuated and flushed repeatedly and then stored at 60 cm Hg positive pressure with prepurified nitrogen for 5 days prior to irradiation.

NI immediately after irradiation seeds were soaked in distilled water and germinated.

NSN after irradiation seed ampoules were stored unopened for 5 days prior to germination.

NSO immediately after irradiation seed ampoules were opened and placed in a pressure bomb and stored 5 days in oxygen at 200 pounds pressure prior to germination. This is approximately 65 times the concentration of oxygen in air.

The doses (in kr) giving 50% reduction in height relative to specific controls as estimated from this plot are:

	HD_{50}	Relative Sensitivity
NI	41	1
NSN	30	1.4
NSO	7	5.9

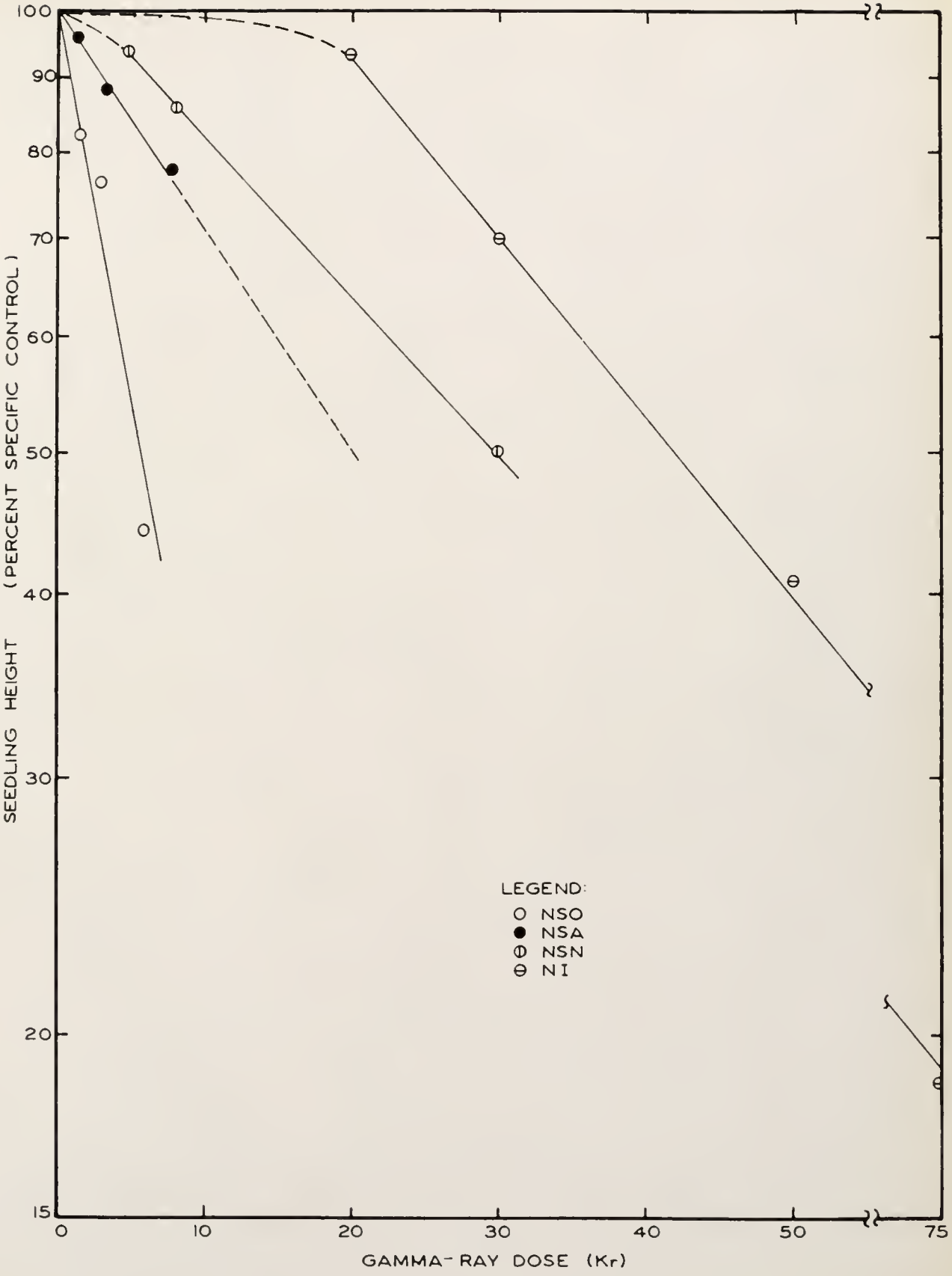

SEEDLING HEIGHT (PERCENT SPECIFIC CONTROL)
100
90
80
70
60
50
40
30
20
15
0
10
20
30
40
50
75
GAMMA-RAY DOSE (Kr)
LEGEND:
NSO
NSA
NSN
NI

FIGURE 13

REGRESSION OF SEEDLING HEIGHT ON PER CENT NORMAL METAPHASES FOR SEEDS IRRADIATED IN NITROGEN AND STORED AT DIFFERENT OXYGEN TENSIONS

Details for this experiment are given with Figure 12. Cytological data and statistical computations are presented in Table 7. The data presented here were obtained from seedlings from that dose level for each post-irradiation treatment which gave a mean height between 40 and 50% of that of the pooled controls. Each point represents the per cent normal metaphases for a single root tip. The data representing pairs of roots from a single seedling are connected by a vertical line. The data from Table 7 are summarized below:

Treatment	Dose (kr)	Mean Height as Per Cent of Control	Regression Equation
NI	50	40.2	$\hat{Y} = 31.16 + 1.53X$
NSN	30	49.3	$\hat{Y} = 30.03 + 1.21X$
NSO	6	49.1	$\hat{Y} = 31.62 + 1.09X$

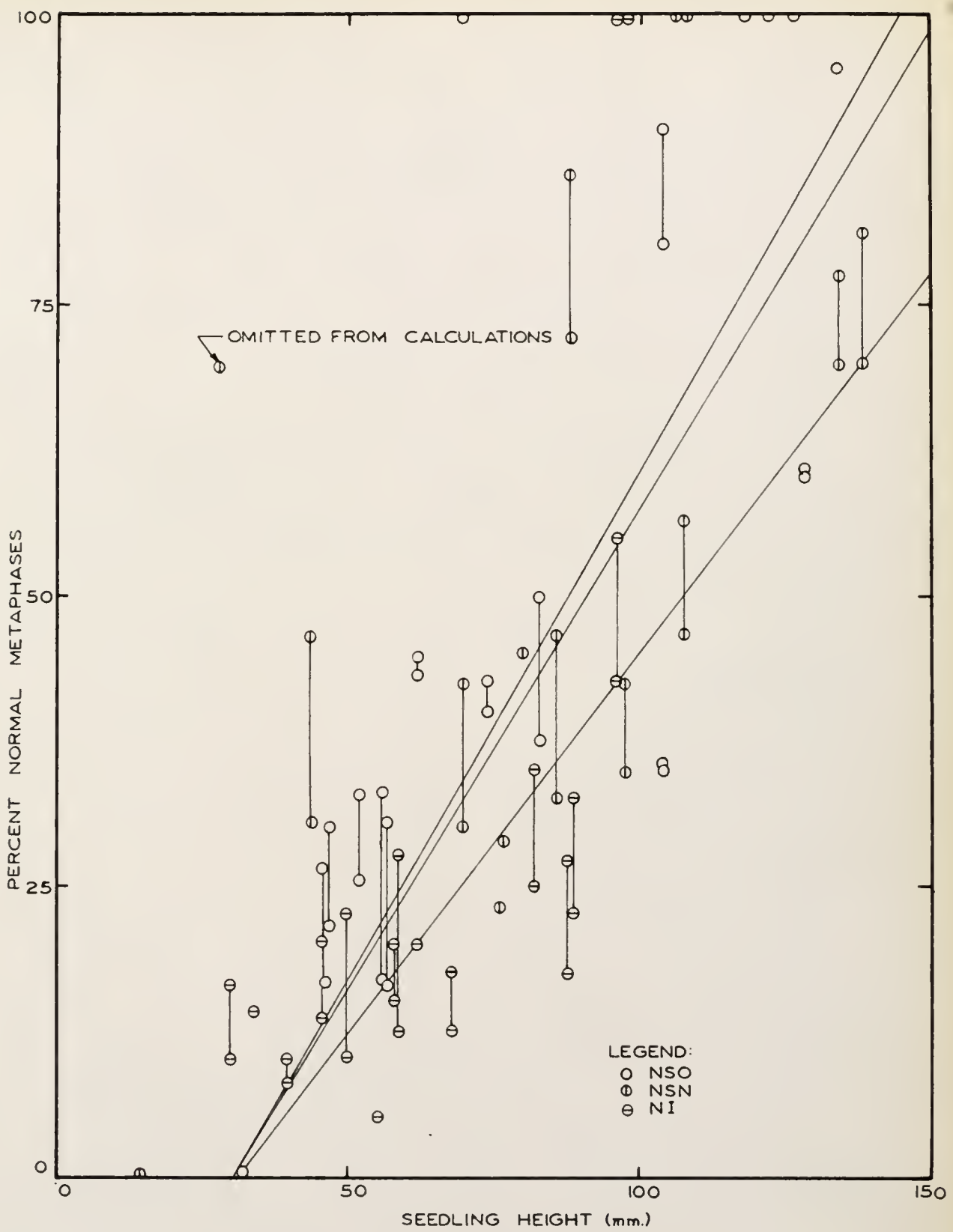

100
75
50
25
0
PERCENT NORMAL METAPHASES
OMITTED FROM CALCULATIONS
LEGEND:
NSO
NSN
NI
0
50
100
150
SEEDLING HEIGHT (mm.)

TABLE 7

SEEDLING HEIGHT AND PER CENT NORMAL METAPHASES FOR SEEDS GIVEN VARIOUS POST-IRRADIATION TREATMENTS, WITH SUMMARY OF CORRELATION ANALYSIS

Seeds with 3.8% water content gamma-irradiated in nitrogen and stored 5 days post-irradiation in oxygen at 200 pounds pressure (NSO) or nitrogen (NSN) or germinated immediately (NI). Seedlings measured at 9 days.

Treatment	NSO		NSN		NI	
Dose	6 kr		30 kr		50 kr	
	Y Height (mm)	X $\bar{x}$ Per Cent Normal	Y Height (mm)	X $\bar{x}$ Per Cent Normal	Y Height (mm)	X $\bar{x}$ Per Cent Normal
	134	95*	138	76	97	49
	132	60	134	74	89	28
	106	35	108	52	88	22
	104	85	98	39	82	30
	83	44	88	80	68	15
	74	41	86	40	62	20*
	62	44	80	45*	58	20
	57	24	77	29	58	18
	57	25	76	23	56	5
	52	30	70	26	50	16
	47	22	57	28*	46	17
	47	26	44	39	40	9
	32	0	14	0	34	14
					30	13
Sum	987	531	1070	561	858	276
Mean	75.9	40.8	82.3	43.2	61.3	19.7
Treatment Mean	49.1		49.3		40.2	
Regressions Height on % Normal	$\hat{Y} = 31.62 + 1.09X$		$\hat{Y} = 30.03 + 1.21X$		$\hat{Y} = 31.16 + 1.53X$	
% Normal on Height	$\hat{X} = -9.85 + 0.6673Y$		$\hat{X} = -2.14 + 0.5509Y$		$\hat{X} = -4.3 + 0.3916Y$	
Correlation Coefficient	$r_1 = 0.8513$		$r_2 = 0.8154$		$r_3 = 0.7732$	
	$z_1 = 1.262$		$z_2 = 1.143$		$z_3 = 1.028$	
	$s_{\bar{d}_{1,2}} = 0.447$		$t = 0.266$	$d.f. = \infty$		$P > 0.8$
	$s_{\bar{d}_{2,3}} = 0.4369$		$t = 0.262$	$d.f. = \infty$		$P > 0.8$
	$s_{\bar{d}_{1,3}} = 0.4369$		$t = 0.533$	$d.f. = \infty$		$P > 0.5$

*Single root examined cytologically

It became evident during the course of the observations, however, that there exists a definite inverse relationship between per cent normal metaphases and average number of aberrations per cell. Tall plants had few abnormal metaphases and these usually consisted of only a single fragment. They rarely had aberrations resulting from multiple breaks. Conversely, many multiple break aberrations (e. g. rings, dicentrics, occasional tricentrics, and translocations) as well as a high number of fragments were present in short individuals which had few or no normal metaphases regardless of dose.

One abnormality observed only rarely was an apparent lack of synchrony indicated by the presence of one pair of daughter chromosomes in an extended state (2 to 4 times the length expected by comparison with the other chromosomes) and with little matrix material. These "extended" chromosomes characteristically are separated by some distance from the others in the squash preparations, but this may be an artifact resulting from pressing the cells. Whether these chromosomes represent a precocious loss of matrix and uncoiling, or the failure to attain normal metaphase development is unknown; however, kinetochore separation had occurred in all the cases observed. This would tend to support the former hypothesis. Records were not kept initially, but later data indicate a frequency of this abnormality in irradiated cells of 0.00015. None was observed in controls.

It should be noted that the barley used in these experiments is remarkably free from chromosomal abnormalities for all the treatments used in these investigations except irradiation. Only two aberrations were observed in approximately seven thousand cells examined from control plants.

DISCUSSION

It was recognized at the meeting held in Karlsruhe, on the effects of ionizing radiations on seeds, (Sparrow, 1961, p. 646) that one of the pressing needs in the area of seed irradiation investigations is, if not a standardization of method, at least an itemization of the numerous variables involved in each experiment so that results can be evaluated properly and comparisons drawn. As yet the bill of particulars promised has not been published. It is with this in mind that the following comments are made.

Methodology

The barley seed supply, at least for most workers in this country, is fairly uniform. Konzak, Nilan, Caldecott, Curtis, Conger, Wolff, and others all use the same selected strain of the variety Himalaya. However, even within this carefully selected and hand-harvested material there is considerable variation. An occasional harvest results in poor germinability (Konzak, 1963). Seed stored less than eight months or more than three or four years is somewhat unreliable and the conditions of storage (temperature and humidity) can influence both germination and radiosensitivity (cf. Davidson, 1960).

Even after careful screening and the removal of obviously defective seeds the weight of individual seeds used in these experiments ranged from about 0.025 g to 0.055 g, a twofold difference, when stored over P_2O_5. Wetter seeds should vary even more. Much of this admittedly

reflects variation in endosperm rather than embryo size since the embryo represents only about 3% of total weight, but it is one source of variability.

If the caryopses are not soaked thoroughly in water, preferably by submersion and mild aspiration prior to sowing on wet blotting paper, their germination is slower and more erratic.

If the seed is sown with the embryo turned down and if there is more than a thin film of moisture on the blotting paper (i. e. if the embryo is actually submerged) the seedling may be delayed a day in germinating.

In the very dry seeds, particularly, any rough handling can readily fracture the embryo which is very brittle. An occasional seed, approximately one in two or three hundred, encounters mechanical difficulty in rupturing the pericarp and the shoot is delayed or prevented from emerging or else carries the endosperm aloft with it, thus depriving itself of nutrition.

These are all minor variables but do contribute to the total variability.

One difficulty in relating experimental results from different laboratories which the writer has encountered, is the lack of any standardized method of determining water content. Some workers grind the seeds; others leave them intact. All, so far as the writer is aware, use some method of drying and estimating original water content on the basis of weight loss. The drying, however, may be in a vacuum oven, or an air oven. The temperature and duration of treatment varies widely (cf. Nilan et al., 1962).

The writer has based his estimates on the procedure of Hart et al. (1959). These workers have developed a simple method of oven drying samples which differs from others such as those of the Association of Official Agricultural Chemists (Horwitz, 1960, p. 169, 158, 124) principally in that the seeds are not ground but are heated whole in roughly 10-gram samples for 20 hours at 130° C. This avoids any unknown or uncontrolled gain or loss of moisture in the grinding process and the time has been set by testing against the presumed highly precise chemical method known as the Karl Fischer method (Hart et al., 1957) from which it gave a mean deviation of -0.01% and standard deviation of 0.29%. The procedure as outlined above was tested on grain of normal moisture. How well it applies to extremes of moisture content, as often used in experiments, is not known. Conger (unpublished) has demonstrated the feasibility of estimating water content of seeds by nuclear magnetic resonance techniques. In any event scientists owe it to their colleagues to specify what method they have used, and yet many do not.

One persistent source of methodological diversity is the time at which seedling height (length of first leaf) is determined. Moes (1961) measured his plants when the controls were about 75 mm. Wolff (1961) measured his when the first leaf was fully grown. Caldecott and also Konzak at one time measured their seedlings at 14 days (Caldecott et al., 1952; Konzak, 1955) but more recently Caldecott (1961) measured 7 day old seedlings, and Konzak et al. (1960) varied the time from 5 to 11 days depending on the temperature at which the seedlings were grown. Most workers seem to favor 6 to 8 days or a control mean height around 10 to 12 cm. In a test of growth with time, the writer found that for the growth conditions specified in the present work the first leaf on

control plants continued to grow for fourteen days to a maximum of 20-21 cm but growth was exponential only from day two through day eight at which time the maximum length was 16 cm. Plants at dose levels giving about 50% height reduction had essentially completed growth by the seventh day. One must conclude that data based on height relative to control under these diverse conditions can be compared in a quantitative manner only with great difficulty and considerable uncertainty, and not at all in a surprising number of cases where no actual heights are given.

Another misleading practice is the citing of mean heights with no indication of the distribution of the population about the mean. In the principal study dealt with here, that of very dry seeds gamma-irradiated and stored in air, the mean quite regularly falls between two modes and is often one of the least frequently represented portions of the distribution (Figures 1 and 2). Yet most of the data in the literature treats this exceptional material in the same way as that which is more normally distributed. In fact, for cytological purposes, random samples, often quite small, are drawn from these populations and assumed to be truly representative.

Until a few years ago almost all of the cytological data on first division cycle mitoses in irradiated barley grains was on anaphase figures, either in root tips or shoot tips. It was not until Wolff and Luippold (1956) published their method for obtaining numerous well-spread metaphases that metaphase analysis became practical. The discrepancy between metaphase analysis and anaphase analysis (Wolff, 1957) indicates that as dose increases an increasing proportion of aberrations visible at metaphase is no longer discernible at anaphase; hence metaphase analysis is preferable.

Comparison of data

Turning now to results comparable to those of the present work, let us consider first the results with very dry seeds. Caldecott et al. (1952) observed that barley seed "which had been kept in a humidity controlled desiccator for at least three weeks prior to treatment" gave rise to a great range in individual seedling heights when sown after receiving 20 kr of X rays, in contrast to the much more uniform effect of thermal neutrons. Lower doses of X rays gave more normal distribution. Neither the precise water content nor how much time may have intervened between irradiation and germination was stated but the phenomenon of heterogeneity was observed. The cytological data were reported only on an average basis. Apparently an attempt was made (Caldecott and Smith, 1952, p. 141) to obtain cytological data from root tips of plants which were then permitted to grow for seedling observation but this was abandoned as impractical and random sampling was continued as the preferred method.

Curtis et al. (1958) present a photograph (their Figure 1) which clearly shows the great heterogeneity of seedling height response in barley seeds of 4% water content which were x-irradiated and stored for 24 hours in air before soaking and sowing. They do not mention this phenomenon in the text and treat all the data solely as means.

The first approach to considering these widely distributed populations as anything other than normal populations occurs in the paper Caldecott (1961) presented at Karlsruhe. Here he subdivided the population into three height classes: 0 - 5 cm, 5.1 - 9 cm, and 9.1 cm and taller. There was a good inverse relationship between height class and frequency of interchanges in microsporocytes, a lesser one with

mutant X_2 seedling frequency. The results are not strictly comparable to those presented here because the seeds were soaked and sown immediately after irradiation.

The question arises, why should there be so much variation in the response of different individuals to the same treatment? Gustafsson (1937a,b) struggled with this problem many years ago. His hypothesis was that the embryonic nuclei within the seed are not really resting but that vital processes are under way preparatory to their reproduction. A locally high concentration of water would promote greater activity and concomitantly greater radiosensitivity.

His evidence for locally active regions lay in the fact that serial fixations of hydrating seeds resulted in ever increasing proportions of dividing cells. There was no general synchrony to the initiation of division within the root. This he interpreted as indicating that in the "resting" embryo some nuclei are more advanced in their preparations for division and are more radiosensitive.

It is rather difficult to imagine that seeds which have been stored over strong desiccants for as long as a year or which have been evacuated vigorously for a number of hours can have such dissimilar water content that their differential radiosensitivity can be attributed to this cause alone. Ehrenberg (1955b, p. 208) has presented a statistical argument which implicates unequal water content in this differential response, but he also demonstrated a temperature sensitive period in early germination when low temperatures are most injurious.

Bozzini, Caldecott and North (1962) put forward the hypothesis that the variation in response within these treatments of dry seeds, irradiated and stored dry, may be associated with the manner in which specific

critical radiosensitive molecules within the seed give up bound water during dehydration. Although they know of no way to test this hypothesis unequivocally, they do have some supporting evidence. Post-irradiation heat treatments of one to twenty-four hours duration at 75° C completely eliminates the heterogeneity which otherwise occurs, without altering sensitivity to aerobic hydration. This suggests that the heat treatment has induced the same physical state in all radiosensitive sites. This they suggest, may possibly be due to molecular reorientation with the loss of bound water.

In the present study, irradiation of the seeds, at the center of a relatively large distributed-type source of cobalt-60, ensured a uniform dose to the individual embryos. In the context of the reactive site hypothesis, the heterogeneous response observed here with respect to per cent normal metaphases for different individuals within the same radiation treatment must either be ascribed to differences in the number of such sites or to differences in sensitivity of such sites or to both.

Wolff (1963) has obtained data on chromosome exchange frequencies at various moisture levels which he interprets as indicating that in very dry seeds the number of radiosensitive sites does not increase but that sensitivity does. This, he holds, is consistent with the concept that free radicals are produced which have a longer life in the dry system and thus have an increased probability of inducing biological damage.

Osborne et al. (1963), in a survey of the effect of different water contents on radiosensitivity of the seeds of diverse species, conclude that it is characteristic for seeds to show a minimum

radiosensitivity at some intermediate moisture content and to increase in sensitivity at either extreme. In barley this effect has been reported by some workers (Ehrenberg, 1955b) and not by others (Caldecott, 1955a,b). In the present work there was a suggestion that there is such a minimum but the humidity at which this was achieved was not one of the routine ones being studied so the data are from one experiment only. Figure 9 compares the present observation with those of Ehrenberg (loc. cit.). Ohba (1961) found Japanese red pine seed least sensitive at 13% water content.

An explanation for this effect, if it should prove consistent, can be based on the free radical hypothesis as reviewed by Osborne in the aforementioned paper. At low water concentrations radicals have a high probability of interaction with biologically significant molecules since relatively few competing water molecules are present. As water concentration increases more harmless, radical-radical and radical-water "nullifying reactions" occur until a point is reached where, although radical decay is rapid, the increase in radicals formed, their greater proximity to critical molecules, andtheir possibly greater mobility more than offsets the harmless "nullifying reactions" and sensitivity once more increases.

In the case of post-irradiation treatment with oxygen immediately following irradiation in nitrogen (NSO) the HD_{50} (dose required to reduce height by 50%) obtained in the experiment illustrated by Figure 11 was 5 kr while the HD_{50} for the seeds left in nitrogen (NSN) was 30 kr. This sixfold increase is comparable to that reported in the literature (e. g. Nilan et al., 1961). In two other experiments the respective values were 2.5 to 17.5 and 2.5 to 8.5 giving a 7-fold and a 3.4-fold

"oxygen effect" during storage. This type of fluctuation from experiment to experiment is typical of that encountered and suggests that there may be several variables which are not being controlled.

Additional confirmation that there is a direct relationship between the frequency of aberrations in root tips and various other criteria of radiosensitivity has come from widely separated laboratories and with other varieties of barley. Ivanov and Kalikov (1960) tested a number of varieties of both wheat and barley and found the relationship to hold. Yanushkevich (1961), while finding a similar relationship, could not relate radiosensitivity to age, maturity, storage or water content, but, rather, associated it with locality of cultivation. Avanzi (1960) in studies of both shoot and root from the same plants found that with irradiation there was close agreement in growth inhibition, but that plants averaging 10% growth inhibition in both organs had four times as many chromosome breaks in the shoot as in the root.

It becomes clear that full understanding of the interrelations between the many observable consequences of radiation treatments of such a complex biological system as that of barley grain must await the identification of and carefully controlled experimentation with each and every significant variable. That day is fast approaching!

SUMMARY

Barley grains were irradiated at a central location within a distributed-type cobalt-60 gamma source at a dose rate of approximately 4,000 r per minute for total doses of one to 75 kr. The effects of moisture content from 2.8 to 15.5% water (by weight) and different oxygen tensions, during and after irradiation, upon radiosensitivity were determined. The criteria of radiation damage were: 1, reduction in growth of the first seedling leaf with respect to controls and 2, the per cent of cells showing no detectable chromosomal aberrations when arrested, by colchicine, at metaphase of the first division cycle in root tips. These observations were made on the same individuals.

Results demonstrate that excision of two root tips from day old seedlings has no significant effect on subsequent growth. Comparison of the cytological data obtained from the two related roots shows that a close correspondence usually exists, the difference in per cent normal metaphases being about seven on the average.

There is a high positive correlation ($r > 0.85$) between seedling height and per cent normal metaphases for all conditions that were examined. These were as follows:

1. Seeds of 2.8% water content irradiated in air or oxygen with doses from one to 36 kr and stored at least five days;
2. Seeds with 3.8, 6.7, 7.3, 9.2, 10.1, 12.8 and 15.5% water content irradiated in air with doses up to 50 kr and stored at the same water content in air at least six days;

3. Seeds with 3.8% water content evacuated and stored at least one day under slight positive pressure of nitrogen; irradiated in nitrogen with doses up to 75 kr and either germinated immediately, or stored for at least four days in nitrogen, or air, or in oxygen under moderate positive pressure.

The high positive correlation between seedling height and per cent normal metaphases holds true for individuals within a treatment as well as for mean values between treatments. This response of individuals within treatments was the subject for special study with very dry seeds (less than 3% water content) where great diversity in leaf length within treatments has been generally observed.

The writer concludes that the variable extent of chromosomal damage among individuals within treatments is a sufficient cause for the heterogeneous growth response. This great diversity can not be attributed to variation in the dose absorbed. The "direct effect" must therefore be relatively uniform. Evidence for "indirect effect" has been amply demonstrated by other workers and is apparent from post-irradiation modification experiments reported here. The present study suggests that indirect effect on seedling height is mediated through the chromosomes, and that, for it to be modified, there must be either protection or repair of the chromosomes themselves.

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VITA

Harlan Quinn Stevenson, son of Wilbur Harlan and Roberta Quinn Stevenson, was born April 1, 1927, and raised on a farm at Midvale, Pennsylvania. He attended public elementary school at Rouzerville and high school at Waynesboro, Pennsylvania. He enlisted in the United States Naval Reserve March, 1945, and served seventeen months active duty principally in the Philippines.

He attended college at Pennsylvania State University, receiving the degree of Bachelor of Science in Science, February, 1950, and immediately commenced graduate study in Botany at that University, holding a graduate teaching assistantship for three semesters. He transferred September, 1951, to Cornell University where he again held a graduate teaching assistantship in Botany while majoring in Cytogenetics.

September, 1955, he took employment at Brookhaven National Laboratory as an Associate in Biology. Several papers were published in collaboration with Dr. Harold H. Smith as a result of work done there. In September, 1960, he entered the University of Florida as a graduate student in Botany with a major in Radiation Biology. He was recipient of a Graduate School Fellowship 1960-61 and Nuclear Science Fellowship 1961-63.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1963

Dean, College of Agriculture

Dean, Graduate School

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